Centre Africain de Recherches sur Bananiers et Plantains

CARBAP

ETUDE SUR LES CONDITIONS DE REINTRODUCTION DES FONGICIDES SYSTEMIQUES DANS LES PROGRAMMES DE LUTTE CONTRE LA MALADIE DES RAIES NOIRES AU CAMEROUN DANS LA ZONE DE PRODUCTION DE LA BANANE DESSERT D'EXPORTATION

RAPPORT NARRATIF INTERMEDIAIRE N°8
Deuxième campagne de monitoring à la CDC/DELMONTE 2010

Contrat de service N°146 – 762/786/798/801(Cris)

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Josué Essoh Ngando, Luc de Lapeyre de Bellaire

Avec la collaboration technique **de F. Tchipe, J. Essome, S. Kana, C. Essoh et H. Mpouli, Robert Dongmo et Oscar Nguidjo**

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Bureau de liaison B.P. 832 Douala-Cameroun – Tel : (237) 99964094 - Tél/Fax.: (237) 33425786 – Centre de recherche : Njombé. Tel. : (237) 33060329
E-mail : carbap@carbap-africa.org. Site web : www. carbapafrica.org

Description

1.1. **<u>Nom du bénéficiaire du contrat de subvention</u>:**
Centre Africain de Recherches sur bananiers et Plantains (CARBAP)

1.2. **Nom et fonction de la personne de contact :**
Jean Daniel Ngou Ngoupayou, Directeur du centre

1.3. **<u>Nom des partenaires de l'Action</u>:**
CIRAD (Centre International de Recherches Agronomiques pour le Développement) et Bayer CropScience

1.4. **<u>Intitulé de l'Action</u>:**
Etude sur les conditions de réintroduction des fongicides systémiques dans les programmes de lutte contre la Maladie des Raies Noires au Cameroun

1.5. **<u>Numéro du contrat</u>:**
N° 146 – 762/786/798/801 (Cris)

1.6. **<u>Date de début</u> et <u>date de fin</u> de la période de reporting:**
Octobre à novembre 2009

1.7. **<u>Pays</u> ou <u>région(s)</u> cible(s):**
Cameroun

1.8. **<u>Bénéficiaires finaux</u> et/ou <u>groupes cibles</u>[1] (si différents) (y inclus le nombre de femmes et d'hommes):**
Plantations agro Industrielles de bananes destinées à l'export

1.9. **Pays dans lequel/lesquels les activités sont réalisées (si différent du point 1.7):**

2. Evaluation de la mise en œuvre des activités de l'Action

2.1. Rappel du contexte de l'étude

La maladie des raies noires (MRN) est la principale contrainte parasitaire des plantations agro-industrielles de bananes dessert. Cette maladie foliaire, présente dans la majeure partie des zones de production de bananes dans le monde, est provoquée par le champignon ascomycète et aérien *Mycosphaerella fijiensis*. Les attaques de ce champignon peuvent entraîner une réduction de l'activité photosynthétique et des pertes de rendement variant de 10 à 100%. Toutefois, l'effet le plus important de la maladie est indirect car les régimes récoltés sur les plants fortement affectés ont une durée de conservation fortement réduite et ne peuvent donc pas être exportés. En l'absence de variétés résistantes (non disponibles à ce jour), la culture intensive de la banane dessert pour l'export n'est donc réalisable qu'au moyen d'un contrôle chimique rigoureux de cette maladie. Au Cameroun, *M. fijiensis* a été signalé pour la première fois en 1981. A la fin des années 80, une méthode d'avertissement utilisant des descripteurs biologiques a été mise au point et appliquée avec succès, limitant ainsi le nombre d'applications à 12-14 par an. Cette lutte raisonnée par avertissement reposait fortement sur l'emploi de fongicides systémiques ayant un fort effet curatif. Malheureusement, depuis 1996 l'apparition de souches résistantes aux fongicides systémiques a entraîné l'abandon de cette stratégie au détriment d'une méthode de lutte plus systématique reposant majoritairement sur l'emploi de fongicides de contact. Les fongicides de contact ne provoquent pas l'apparition de souches résistantes, mais ils n'ont pas d'effet curatif sur la maladie, et sont donc utilisés préventivement. Ainsi, en 2006, malgré un souci constant de continuer à piloter la lutte chimique par l'observation de descripteurs biologiques, environ 40 traitements ont été effectués sur la majorité des plantations. Cette augmentation du nombre de traitements a entrainé une augmentation du coût de la lutte, mais également des risques environnementaux. En effet, en plus de l'augmentation des quantités de matière active liées à l'accroissement du nombre de traitements, les fongicides de contact sont épandus à des doses plus importantes que les fongicides systémiques. De nouvelles stratégies de traitement doivent être aujourd'hui redéfinies pour retrouver une situation plus durable sur les plans économiques et environnementaux

2.2. Rappel des objectifs de l'étude et de la méthodologie

2.2.1. Objectifs

Les observations récentes des derniers monitoring montrent qu'il y a une baisse des niveaux de résistance dans certaines plantations commerciales du Cameroun, plus particulièrement depuis que les fongicides systémiques ne sont plus ou peu employés. Cette évolution permet de penser que les phénomènes de résistance aux fongicides sont peut être réversibles.
Plusieurs mécanismes peuvent être à l'origine de cette évolution récente des niveaux de résistance :

- des flux de gènes provenant des zones non traitées (effectifs élevés de populations sensibles aux fongicides), vers les plantations commerciales (effectifs faibles de populations résistantes) qui pourraient entrainer une « dilution » progressive du phénomène de résistance
- une perte de compétitivité des souches résistantes qui seraient alors progressivement éliminées lorsque la pression de sélection fongicide est arrêtée (plus de traitements avec des fongicides systémiques)

L'objectif de cette étude est ainsi de :

- Mesurer l'évolution dans le temps du niveau de résistance aux fongicides systémiques dans les populations pathogènes de *M. fijiensis* des plantations industrielles du Cameroun.
- Définir les conditions d'un réemploi éventuel des fongicides systémiques dans le cadre des différentes stratégies de traitement utilisées au Cameroun. Plus particulièrement est visée la possibilité de réutiliser des stratégies de traitement basées sur un système d'avertissement.

2.2.2. Méthodologie

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Le programme de travail de cette étude a été regroupé en 5 activités spécifiques qui permettront de répondre aux objectifs de l'étude :

Activité spécifique 1. Améliorer les méthodes d'évaluation de la résistance aux fongicides.

Activité spécifique 2. Evaluer les niveaux de résistance dans les différentes plantations commerciales du Cameroun.

Activité spécifique 3. Mesurer les flux de gènes entre les plantations non traitées et les plantations commerciales.

Activité spécifique 4. Mesurer l'impact de stratégies de traitement sur la résistance aux fongicides

Activité spécifique 5. Evaluer la compétitivité des souches résistantes par rapport aux souches sensibles

2.3. Summary of the action

During this period, activities have concerned the second campaign of evaluation of resistance level in CDC/Delmonte plantation, realised from October to November 2009. This evaluation was realised on 8 sectors of plantation and 3 triazole fungicides: BAYCOR 500 SC (bitertanol), OPAL 75 EC (epoxyconazole) and SICO 250 EC (difenoconazole). During the same year, a test of comparison between two resistance methods using two different types of biological materials (ascospores or conidia) were realised.

2.4. Activities and results

2.4.1. Introduction

In order to evaluate the possible reintroduction of the forecasting strategies based on the use of systemic fungicide, it is important to continuously monitor the evolution of the sensitivity of fungal populations inside industrial plantations of Cameroon.

In this project, this monitoring was realised by CARBAP on many sectors of CDC/Delmonte plantation. Ninety (90) analyses are expected by year in this plantation. One part of these analyses corresponding to 66 analyses have been realised at two periods of the year with the common ascosporic method on a certain number of sectors and fungicides defined together with the technical plantation manager (39 analyses were realized in the first monitoring; and 24 in this monitoring). The second part of analyses is realised by the comparison of two different methods (24 analyses): ascosporic and conidial methods on 3 sectors and for all the group of systemic fungicides (benzimidazoles, triazoles and strobilurines). This comparison has been replicated 3 times. Fifty four (54) analyses were realized in this campaign.

So, 114 analyses were realized in this plantation, instead of 90 that were expected.

a. For the comparison of the ascosporic and conidial method - 54 analyses

Sectors	Number of analyses	Fungicides
Tiko	9 + 9	Bankit, Tilt, Callis
Ekona	9 + 9	
mussaka	9 + 9	

b. for analyses with ascosporic method : 24 analyses

Sectors	Number of analyses	Fungicides
Tiko	3	Baycor, Sico, Opal
Ekona	3	
Mussaka	2	

2.4.2. Protocol of the ascosporic method

i) Leaf sampling

Leaf samples were collected from industrial plantations which apply fungicides on a regular basis (farm samples) and from smallholders' plots where fungicides have never been applied (baseline sample).
Leaf samples consist of necrotic tissue where disease symptoms had reached grade 6 (necrotic leaf area with spots showing a clear grey centre).
For each field necrotic tissues of at least 20 banana trees were collected.

i-1) Farm samples:
Leaf samples were collected from October to November 2009 in 9 different sectors of the plantation.

- Tiko

Sample collected	Fungicide tested
Mondoni 2 F7C21 Moquo F3C26 Mondoni 1 F5C3	Sico, Baycor and Opal Tilt, Callis and Bankit

- Ekona*

Sample collected	Fungicide tested
Ekona G8/9 Ekona J6 Ekona G8/9	Sico, Baycor and Opal Tilt, Callis and Bankit

*3 sectors were sampled in this farm, but 1 sector did not produce any ascospores and could not yield any result

- Mussaka

Sample collected	Fungicide tested
Mussaka F1C13 Mussaka F5C19	Sico, Baycor and Opal
Mussaka F5C19	Tilt, Callis and Bankit

i-2) Baseline samples :

For Tiko plantation, samples from smallholder's fields were collected on plantains along the road between Tiko and Mungo bridge.

For Ekona and Mussaka plantation, samples from smallholder's fields were collected on plantains along the road between Ekona and Buea.

ii) <u>Isolation and cultivation of ascospores</u>

- Necrotic leaf samples (containing perithecia) are collected in the plantations.
- After an incubation period of 48 hours at room temperature, leaf fragments were stapled to a disc of about 90 mm diameter of filter paper with the abaxial surface down (lower leaf side down).
- The leaf fragments are submerged for 10 minutes in bi-distilled water and then immediately placed inside the lid of a Petri dish and suspended over the water agar amended with different fungicide concentrations.
- A minimum of 3 hours is needed for discharge of the ascospores.
- Afterwards the filter paper with the attached leaf fragments was removed and the plates were stored at 25 °C for 48 hours.

- The sensitivity of the fungal population was evaluated for the samples collected in different sectors of Del Monte plantations. The fungicides tested were: Sico 250 EC (a.i: difenoconazole), Baycor 300 EC (a.i: bitertanol) and Opal 75 EC (a.i: epoxyconazole).

Different concentrations used for these analyses:

- For Sico: 0 and 0.1 ppm
- For Baycor: 0 and 0.1 ppm
- For Opal: 0 and 0.1 ppm
- For Tilt: 0 and 0.1 ppm
- For Bankit: 0 and 10 ppm
- For Callis: 0 and 5 ppm

iii) <u>Evaluation of fungal growth</u>

- The length of the germ tubes of the ascospores were visually assessed with a microscope.
- Fifty spores were observed per sample for Sico 250 EC, Baycor 300 EC, Opal 75 EC, Tilt and Bankit 25 SC.
- Response to Sico 250 EC, Baycor 300 EC, Opal 75 EC, Tilt and Bankit 25 SC was estimated by examining the germ tube lengths. Germ tube lengths were measured with the aid

of a microscope outfitted with an indexed objective lens. The relationship between the real length and the observed length is expressed through a conversion factor, specified by the microscope manufacturer.

- The results of spore growth are presented as a percentage of the control (ascospores germinating on agar not amended with the fungicide).
- Response to Callis was estimated by examining the morphology of the germ tube, which varied from normal germination to absence of germination. Normal and short germ tube are classified as resistant phenotype and distorted and non germinated are classified as sensitive phenotype.

2.4.3 Results

These results will be presented according to the name of the 3 plantations of CDC (Tiko, Ekona and Mussaka), for better understanding by the companies.

i) Tiko plantation

i-1) Triazoles

- ***Sico 250 EC (difenoconazole) – table 1; figures 1 and 2***

For difenoconazole, the average percentage of growth inhibition (%GI) in the analysed sectors (67-71 %) is close to the baseline sample (82%). A low percentage (2-10%) of the population has an inhibition inferior to 50%, phenotype which is not encountered in the baseline sample (100% strains have an inhibition > 50%).

This shows a very light shift of sensitivity to this fungicide in comparison with the baseline sample. This situation reveals an improvement of the sensitivity to this fungicide in Mondoni and Moquo, as compared with the former monitoring.

- ***Baycor 300 EC (bitertanol) – table 2; figures 3 and 4***

For bitertanol, the average % GI in the analysed sectors (48-61%) is lower than in the baseline sample (76%). Moreover, a significant proportion of the population (10-52%) has an inhibition inferior to 50%. This shows a significant shift in sensitivity to bitertanol in this plantation, the most affected sector being Mondoni 2 F7C21 (%GI = 48; 52% strains with less than 50% inhibition). This situation is comparable to the last monitoring.

- ***Opal 75 EC (epoxyconazole) – Tableau 3; figures 5 and 6***

For epoxyconazole, the average % GI in the analysed sectors (64-72%) is relatively close to the baseline sample (79%). A low or unsignificant proportion of the population (0-18%) has an inhibition inferior to 50%. This shows that the sensitivity is close to the baseline for epoxyconazole in this plantation, this situation being comparable to previous data.

- ***Tilt 250 EC (propiconazole) - Tableau 4; figures 7 and 8***

For propiconazole, the average %GI for the only sector (39 or 41%, according to the method) is significantly lower than in the baseline (83%). A significant proportion of the population (55-59 % according to the method) has an inhibition inferior to 50%. This shows a strong shift in sensitivity to propiconazole in this plantation. As compared to the previous data, the sensitivity to propiconazole in this plantation is still decreasing.

The general tendency shows a decreasing sensitivity to triazoles. This state is particularly characterised by a strong shift in sensitivity to propiconazole in spite of the withdrawal of this fungicide. This means that, selection with this group of fungicides is continuing. This shift is unequal for the different fungicides of the triazole family: it is important for bitertanol, , while for difenoconazole and epoxyconazole this shift is slight.

i-2) Strobilurines

- ***Bankit 25 SC (azoxystrobine) - Tableau 5; figures 9 & 10***

For azoxystrobine, the average percentage of inhibition of sector analysed (86-95 % according to the method) is close to the baseline sample (100%). However, a very low proportion of the average population (0-8% for the ascospores method) had an inhibition inferior to 50%, phenotype which is not encountered in the baseline sample (100% strains have an inhibition > 50%). These strains could probably be resistant to this fungicides, nevertheless they were not detected with the conidial method. This indicates the potential presence of few resistant strains in this plantation. Compared with previous data, it is the first time to detect some strains potentially resistant.

This monitoring is marked by the detection of the first strains potentially resistant, but their frequency remains very low. This was not confirmed by conidial method and should be further confirmed.

i-3) Benzimidazole

- ***Callis 400 OL (Methyl-thiophante) – Table 6; figure 11***

For methyl-thiophanate, no resistant strains were detected with the ascospores method, and 2% resistant strains were detected in one replicate of the conidial method and were not detected in the two other replicates.

The sensitivity to methyl-thiophanate remains high even if a very low proportion of resistant strains is sometimes detected, situation that has already been observed in this farm

i-4) Conclusions and recommendations for Tiko farm

Triazoles

A shift in sensitivity to Triazoles is observed in Tiko farm, and this shift is variable according to the fungicides in this plantation : more pronounced for propiconazole and also for bitertanol; light for difenoconazole and epoxyconazole. It is important to note that all the fungicides of the triazole family are actually concerned by this shift, even if it is at different levels.

Frac recommendations is the following: not more than 8 applications should be done with this family of fungicides which include the following products: Tilt, Folicur, Baycor, Sico, Opal, Punch, Vectra. These fungicides should be fully used in alternation with other mode of actions. Because of the shift in sensitivity observed for Tilt, Sico, Opal, and Baycor, in some sectors of Tiko farm, we recommend to reduce this maximum number to 4 applications of triazole. By another hand, we recommend also to reinforce deleafing management programs in this plantation (a poor quality of deleafing was noticed this year in many sectors) because high population levels can accelerate the selection of resistant strains inside the farms.

Strobilurines

The sensitivity of azoxystrobine is closed to the baseline sample. But analyses should be opened to many sectors to carry out the good overview of this plantation. The first potentially resistant strains were detected but not confirmed by conidial test. Before confirming these results, we recommend to follow Frac advisement:

-Apply QoI fungicides only in mixtures or alternation with other, non-cross resistant modes of action, all partners at recommended effective manufactor's rates. No consecutive QoIs-applications can be applied.

-A maximum of 1 to 2 applications containing QoI fungicides. Nevertheless, we advise to interrupt their use until the confirmation (or not) of the presence of resistant strains.

-Applications containing QoI fungicides should preferably start at the onset of the annual disease progress curve and be applied at times of lower disease pressure.

-Applications have to be separated by at least 3 months of a QoI-free period.

Benzimidazoles

The sensitivity to methyl-thiophanate is good, even if a low percentage of resistant strains has been observed in one replicate. Analyses should be opened to many sectors to carry out the real situation in this plantation. We recommend continuing to follow the current strategies according to last Frac's recommendations (2010):

Benzimidazole fungicides should be applied according to the following guidelines against black sigatoka in banana:

-BCM fungicides have to be applied only in mixtures or in full alternation with other, non-cross resistant modes of action. No consecutive BCM-applications (blocks) can be applied.

- A maximum of 2-3 applications containing BCM fungicides or a maximum of 33% of the total number of sprays can be applied with BCMs.

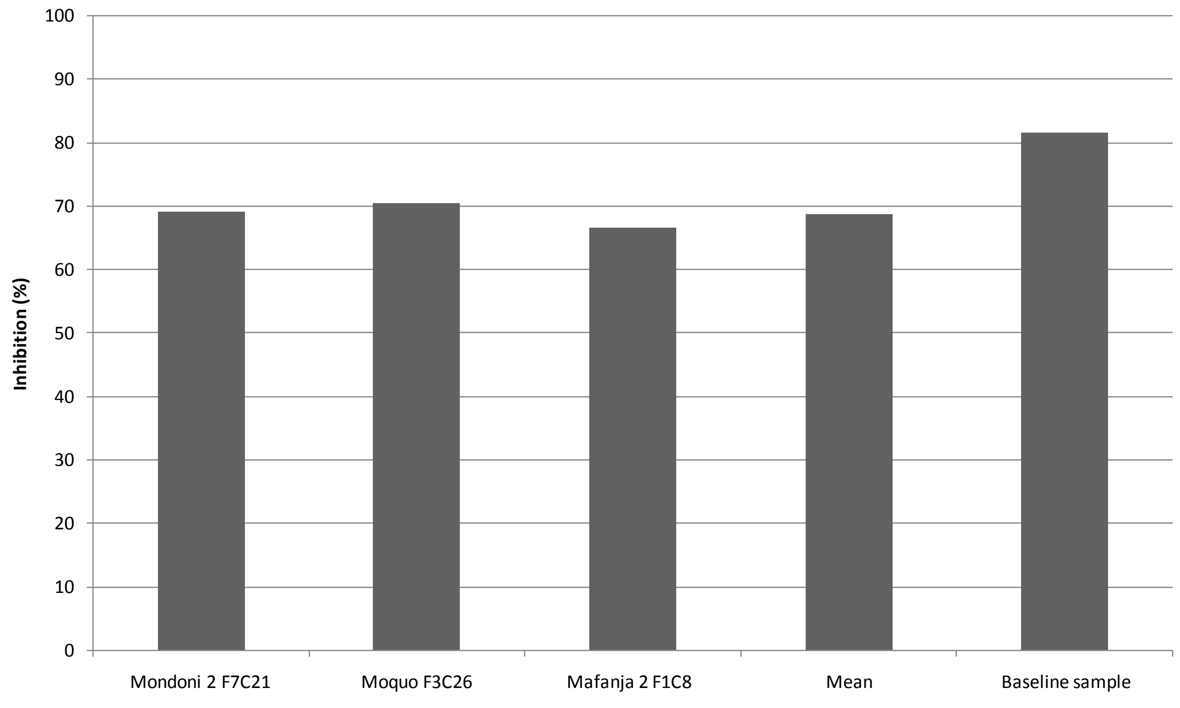
- Applications containing BCM fungicides should preferably start at the onset of the annual disease progress curve and be applied at times of lower disease pressure.

- Applications have to be separated by at least 3 months of a BCM-free period.

<u>Table 1</u>: Summary of results concerning the sensitivity of *M. fijiensis* populations to Sico 250 EC (difenoconazole at 0.1 ppm)

SICO	% inhibition	Lenghts of the germ tubes (µm)	Growth inhibition classes						% spores inhibition < 50 %
Sectors			**0-10**	**11-30**	**31-50**	**51-70**	**71-90**	**91-100**	
Mondoni 2 F7C21	**69**	108	0	0	10	24	66	0	**10**
Moquo F3C26	**71**	96	0	0	2	38	60	0	**2**
Mafanja 2 F1C8	**67**	110	0	2	8	34	56	0	**10**
Mean	**69**	**104**	**0**	**1**	**7**	**32**	**61**	**0**	**7**
Baseline sample	**82**	**56**	**0**	**0**	**0**	**0**	**100**	**0**	**0**

Summary of the results of previous monitoring									
June-09	60	112	1	7	23	22	46	0	31
nov-09	**69**	**104**	**0**	**1**	**7**	**32**	**61**	**0**	**7**



Fiure 1: Percentage of growth inhibition (difenocoazole at 0.1 ppm)

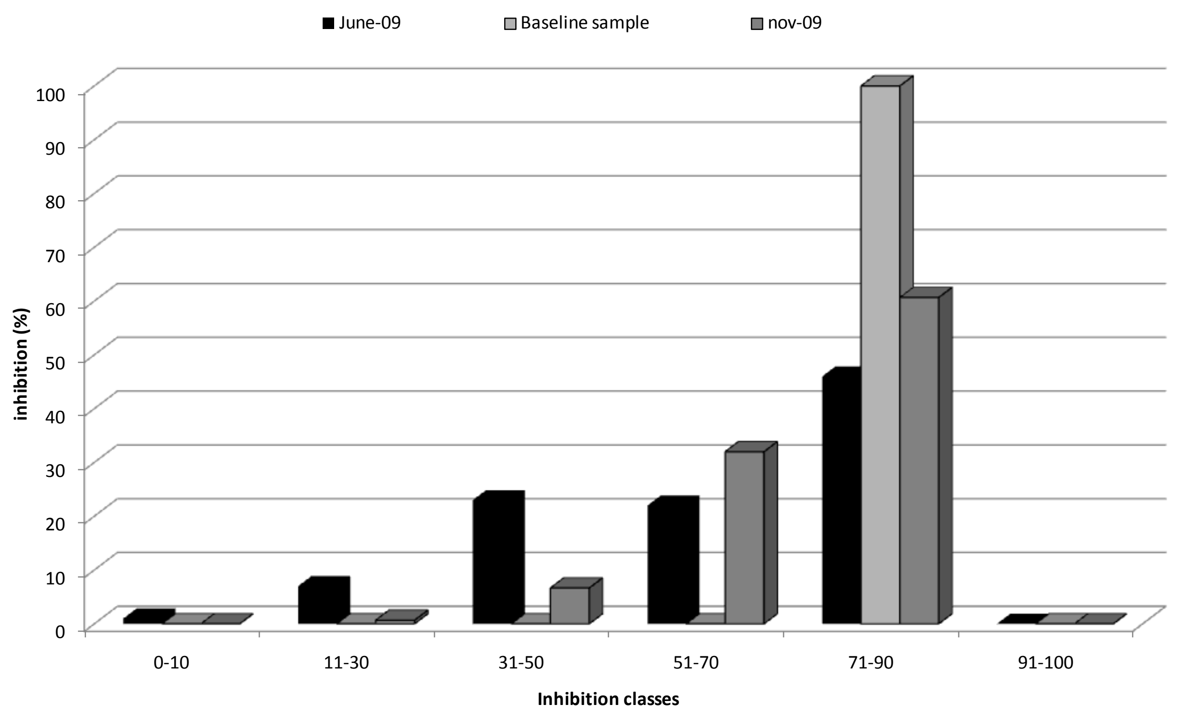


Figure 2: Distribution of spores in inhibition classes (difenoconazole at 0.1 ppm)

<u>Table 2</u>: Summary of results concerning the sensitivity of *M. fijiensis* populations to BAYCOR (bitertanol at 0.1 ppm)

BAYCOR	% inhibition	Lenghts of the germ tubes (µm)	Growth inhibition classes						% spores inhibition > 50 %
Sectors			0-10	11-30	31-50	51-70	71-90	91-100	
Mondoni 2 F7C21	**48**	183	0	16	36	36	12	0	**52**
Moquo F3C26	**61**	127	0	0	14	52	34	0	**14**
Mafanja 2 F1C8	**64**	119	0	4	6	44	46	0	**10**
Mean	**58**	**143**	**0**	**7**	**19**	**44**	**31**	**0**	**25**
Baseline sample	**76**	**73**	**0**	**0**	**0**	**8**	**92**	**0**	**0**

Summary of the results of previous monitoring									
déc-05	**68**	64	0	0	4	59	38	0	**4**
déc-06	**65**	84	0	0	11	51	38	0	**11**
nov-07	**63**	108	0	1	11	58	30	0	**12**
juin-09	**59**	119	1	6	22	40	30	0	**30**
nov-09	**58**	**143**	**0**	**7**	**19**	**44**	**31**	**0**	**25**

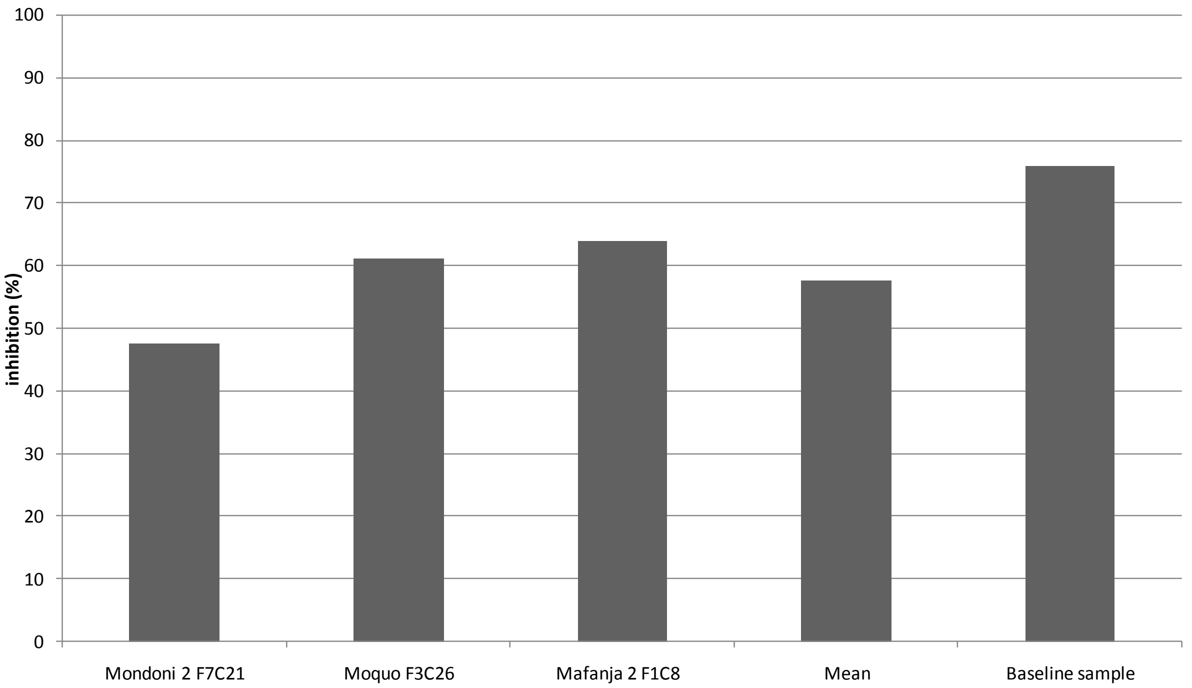


Figure 3: Percentage of growth inhibition (bitertanol at 0.1 ppm)

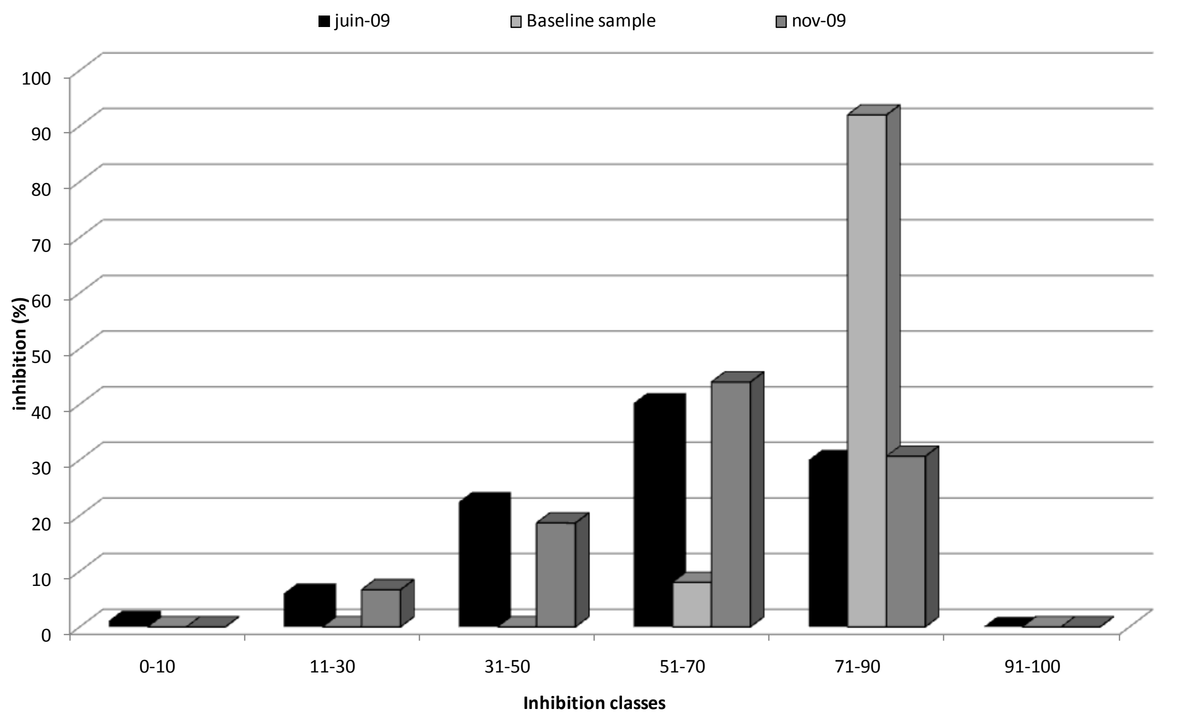


Figure 4: Distribution of spores in growth inhibition classes (bitertanol at 0.1 ppm)

<u>Table 3</u>: Summary of results concerning the sensitivity of *M. fijiensis* populations to Opal 75 EC (epoxyconazole at 0.1 ppm).

OPAL / Sectors	% inhibition	Lenghts of the germ tubes (µm)	Growth inhibition classes 0-10	11-30	31-50	51-70	71-90	91-100	% spores inhibition < 50 %
Mondoni 2 F7C21	**72**	**98**	**0**	**0**	**4**	**32**	**64**	**0**	**4**
Moquo F3C26	**68**	104	0	0	0	56	44	0	**0**
Mafanja 2 F1C8	**64**	118	0	2	16	28	54	0	**18**
Mean	**68**	**107**	**0**	**1**	**7**	**39**	**54**	**0**	**7**
Baseline sample	**79**	**63**	**0**	**0**	**0**	**0**	**100**	**0**	**0**

Summary of the results of previous monitoring

june-09	**71**	84	0	0	9	34	54	2	**9**
nov-09	**68**	**107**	**0**	**1**	**7**	**39**	**54**	**0**	**7**

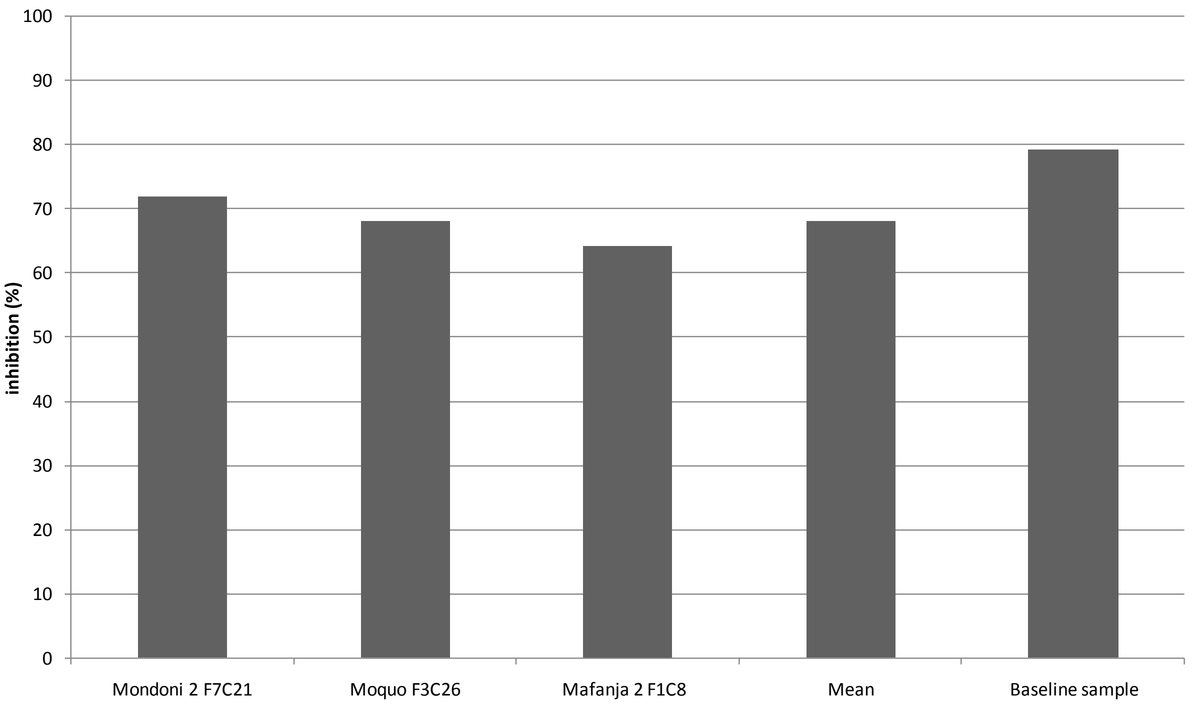


Figure 5: Percentage of growth inhibition (epoxyconazole at 0.1 ppm)

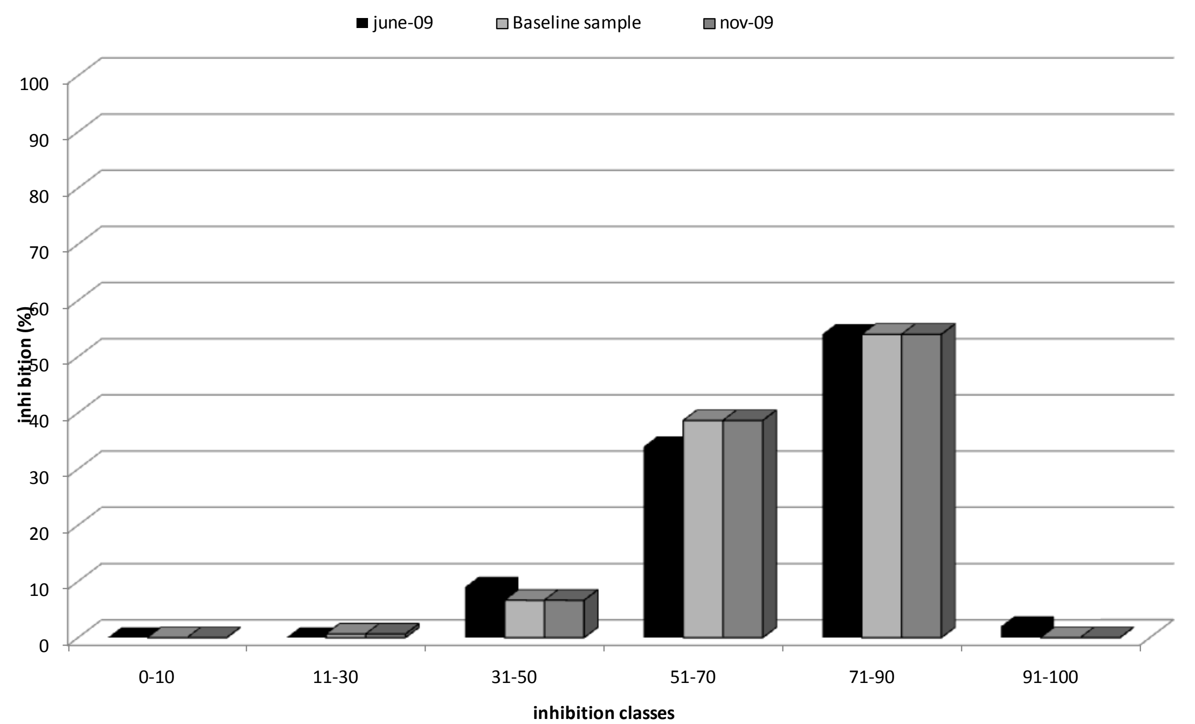


Figure 6: Distribution of spores in the growth inhibition classes (epoxyconazole at 0.1 ppm)

<u>Table 4</u>: Summary of results concerning the sensitivity of *M. fijiensis* populations to Tilt 250 EC (propiconazole at 0.1 ppm).

Tilt	% inhibition	Lenghts of the germ tubes (µm)	Growth inhibition classes						% spores inhibition < 50 %
Sectors			0-10	11-30	31-50	51-70	71-90	91-100	
tiko (asco rep1)	54	152	4	10	20	50	16	0	**34**
tiko (asco rep2)	21	217	50	18	7	20	5	0	**75**
tiko (asco rep3)	42	197	15	10	31	38	6	0	**56**
tiko (conidia rep1)	47	106	15	8	19	42	6	11	**42**
tiko (conidia rep2)	39	103	11	36	28	8	3	14	**75**
tiko (conidia rep3)	36	116	11	25	25	25	9	5	**61**
asco mean	**39**	**189**	**23**	**13**	**19**	**36**	**9**	**0**	**55**
conidia mean	**41**	**109**	**12**	**23**	**24**	**25**	**6**	**10**	**59**
general Mean	**40**	**149**	**18**	**18**	**22**	**30**	**7**	**5**	**57**
Baseline sample	**83**	**51**	**0**	**0**	**0**	**0**	**100**	**0**	**0**
Summary of the results of previous monitoring									
nov-02	**69**	68	0	0	4	4	52	0	**4**
may-02	**72**	73	0	0	1	37	62	0	**1**
may-03	**71**	67	0	0	6	49	52	2	**6**
oct-03	**66**	76	0	0	4	59	37	0	**4**
may-04	**68**	75	0	1	8	41	50	0	**9**
march-05	**66**	71	0	0	14	47	39	0	**14**
déc-05	**52**	98	1	5	34	58	3	0	**39**
nov-09	**40**	**149**	**18**	**18**	**22**	**30**	**7**	**5**	**57**

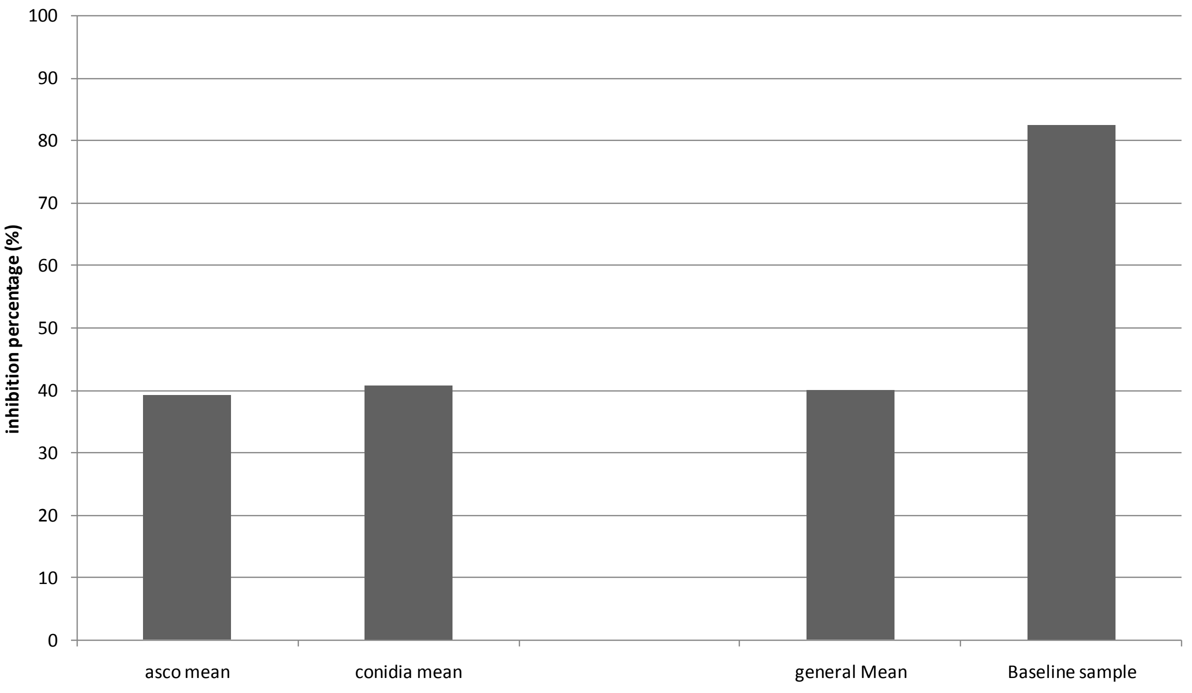


Figure 7: Percentage of growth inhibition (propiconazole at 0.1 ppm)

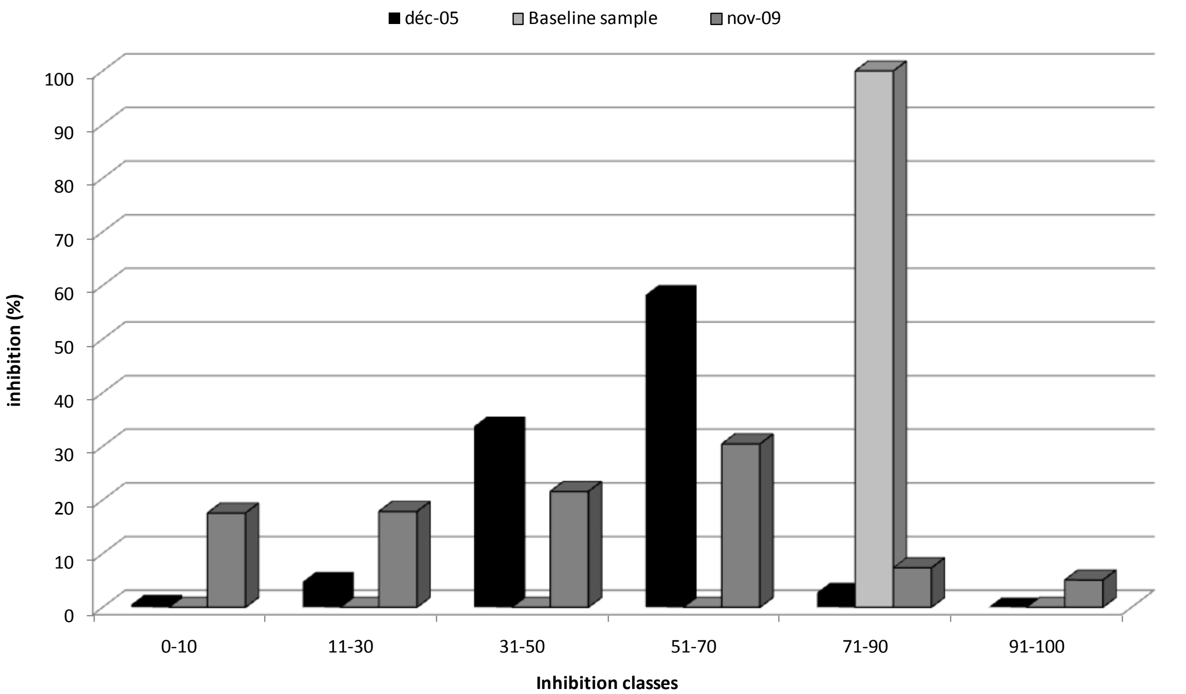


Figure 8: Distribution of spores in the growth inhibition classes (propiconazole at 0.1 ppm)

Table 5: Summary of results concerning the sensitivity of *M. fijiensis* populations to Bankit 25 SC (azoxystrobine at 10 ppm).

Bankit	% inhibition	Lenghts of the germ tubes (µm)	Growth inhibition classes						% spores inhibition < 50 %
Sectors			0-10	11-30	31-50	51-70	71-90	91-100	
tiko (asco rep1)	**87**	36	0	0	8	16	12	64	**8**
tiko (asco rep2)	89	37	0	0	2	18	12	68	**2**
tiko (asco rep3)	82	61	0	0	0	26	32	42	**0**
tiko (conidie rep1)	94	17	0	0	0	4	18	78	**0**
tiko (conidie rep2)	95	15	0	0	0	6	14	80	**0**
tiko (conidie rep3)	96	10	0	0	0	4	10	86	**0**
asco mean	86	45	0	0	3	20	19	58	**3**
conidia mean	95	14	0	0	0	5	14	81	**0**
general Mean	**91**	**29**	**0**	**0**	**2**	**12**	**16**	**70**	**2**
Baseline sample	**100**	**0**	**0**	**0**	**0**	**0**	**0**	**100**	**0**

Summary of the results of previous monitoring									
may-04	**96**	**/**	**0**	**0**	**0**	**0**	**25**	**75**	**0**
march-05	**93**	**22**	**0**	**0**	**0**	**0**	**39**	**60**	**0**
nov-05	**93**	**15**	**0**	**0**	**0**	**1**	**38**	**61**	**0**
nov-09	**91**	**29**	**0**	**0**	**2**	**12**	**16**	**70**	**2**

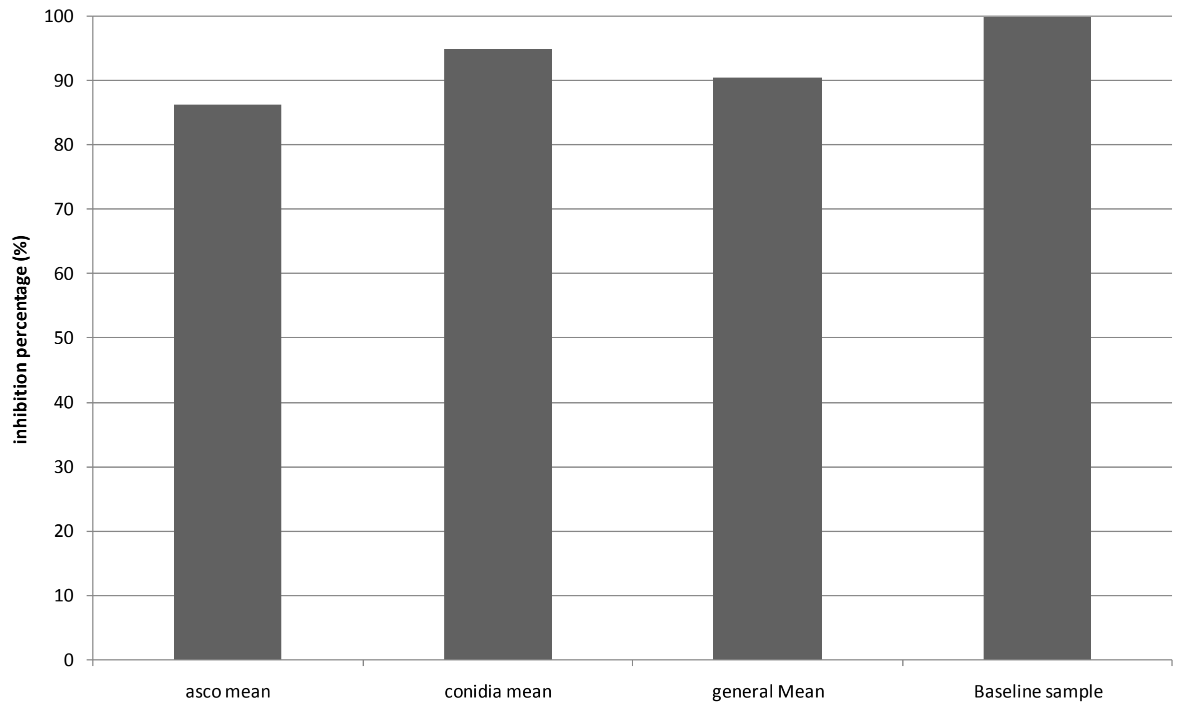


Figure 9: Percentage of growth inhibition (azoxystrobine at 10 ppm)

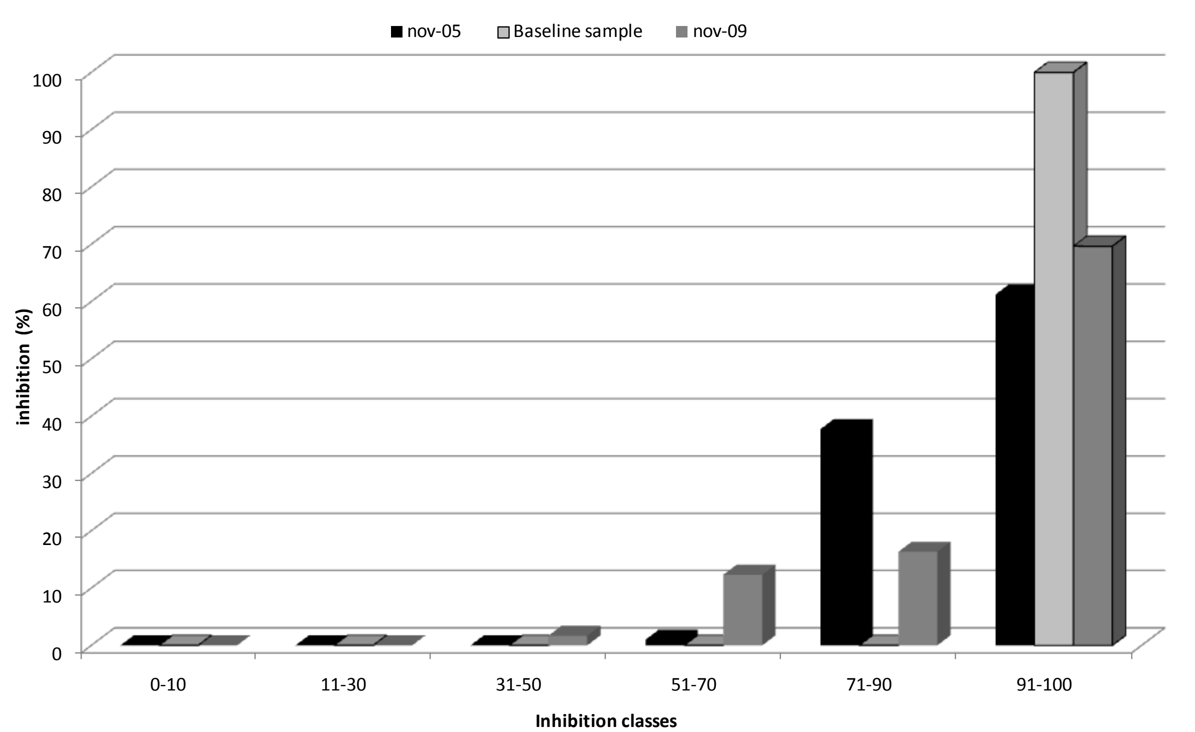


Figure 10: Distribution of growth inhibition (azoxystrobine at 10 ppm)

<u>Table 6</u>: Summary of results concerning the sensitivity of *M. fijiensis* populations to Callis 400 OL (methyl-thiophante at 5 ppm).

CALLIS 400 OL Secteurs	Normal	Disturbed	Short Inhibited	None	% sensitive strains	% resistant strains
tiko (asco rep1	0	54	0	43	**97**	**0**
tiko (asco rep2	0	84	0	16	**100**	**0**
tiko (conidia rep1	0	96	0	4	**100**	**0**
tiko (conidia rep2	2	91	0	7	**98**	**2**
tiko (conidia rep3	0	95	0	5	**100**	**0**
asco mean	0	69	0	30	**99**	**0**
conidia mean	1	94	0	5	**99**	**1**
general mean	0	54	0	43	**97**	**0**
baseline sample	0	100	0	0	**100**	**0**

Summary of the results of previous monitorings						
nov-02	0	96	0	5	**100**	**0**
may-02	19	77	1	3	**80**	**20**
may-03	0	99	0	1	**100**	**0**
oct-03	1	98	0	1	**99**	**1**
may-04	2	92	3	3	**95**	**5**
march-05	0	92	2	6	**98**	**2**
nov-05	0	84	7	6	**90**	**8**
dec-06	0	99	1	0	**99**	**1**
nov-09	0	54	0	43	**97**	**0**

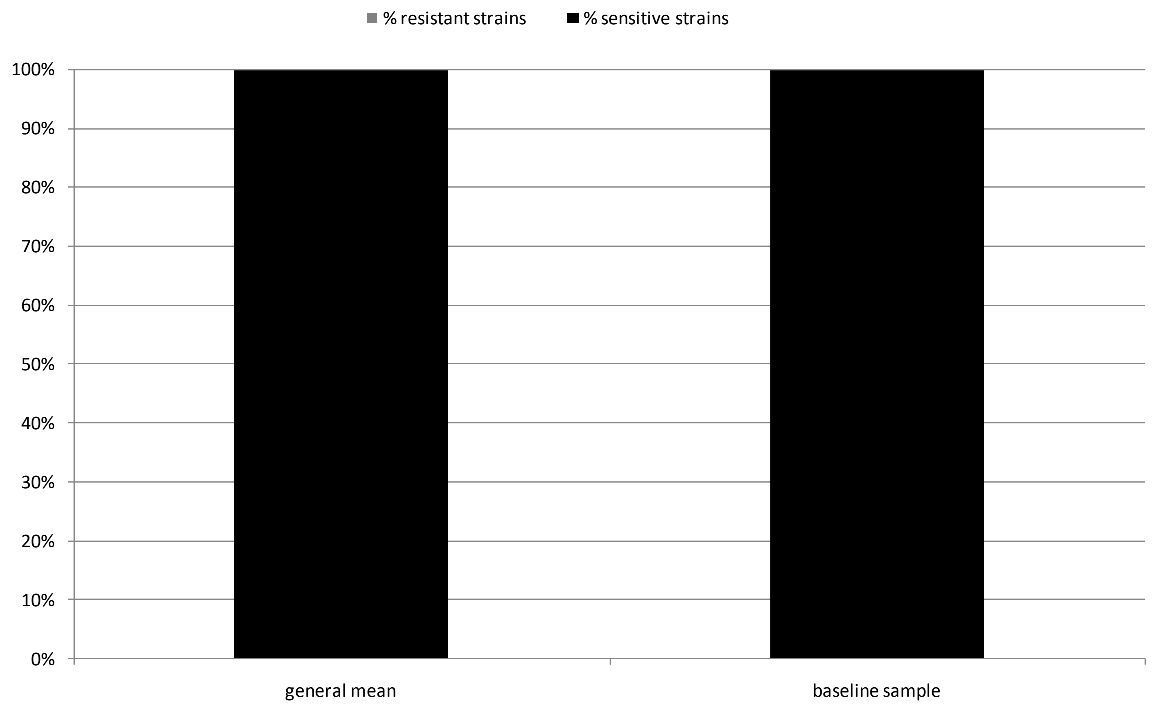


Figure 11: Percentage of sensitive strains (methyl-thiophanate at 5 ppm)

ii) Ekona plantation

ii-1) Triazoles

- ***Sico 250 EC (difenoconazole) - table 1; figures 1 and 2***

For difenoconazole, the average percentage of growth inhibition (%GI) for the 2 different sectors (73-81 %) is close to the baseline (81%). No strains had an inhibition under 50%, phenotype which is not encountered in untreated smallholder populations (100% strains have an inhibition > 50%). This shows no shift in sensitivity to difenoconazole in this plantation. The same situation was observed during the last monitoring.

- ***Baycor 300 EC (bitertanol) – table 2; figures 3 and 4***

For bitertanol, the average % GI for the 2 different sectors (79-80 %) is close to the baseline sample (78%). No strains had an inhibition inferior to 50. This shows no shift in sensitivity to bitertanol in this plantation. The same situation was observed during the two last monitoring.

- ***Opal 75 EC (epoxyconazole) table 3; figures 5 and 6***

For epoxyconazole, the average % GI for the 2 different sectors (61-70 %) is slightly lower than in the baseline sample (79%). A significant proportion of the population (16-28%) has a growth inhibition under 50%. This shows a significant but moderate shift in sensitivity to epoxyconazole in this plantation.

- ***Tilt 250 EC (propiconazole) table 4; figures 7 and 8***

For propiconazole, the average % GI for the sector analysed (24-44 % according to the method) is significantly lower than in the baseline sample (76%). An important proportion of the population (61-82 % according to the method) had a GI < 50% (% according to the method). A strong shift in sensitivity to this fungicide is observed in Ekona farm, situation already observed in November 2007.

A shift in sensitivity to the triazoles has been observed in Ekona farm and is variable according to the fungicides: this shift is very pronounced for propiconazole; moderate for epoxyconazole; no shift was observed for difenoconazole and bitertanol.

ii-2) Strobilurines

- ***Bankit 25 SC (azoxystrobine) - Tableau 5; figures 9 & 10***

For azoxystrobine, the average % GI on the sector analysed (98-100 % according to the method) is equal to the baseline sample (100%). No strains had a growth inhibition lower than 50%, phenotype which is not encountered in untreated smallholder populations (100% strains have an inhibition > 50%)..

As compared to the previous data, the presence of resistant strains to strobilurines is not suspected in Ekona farm

ii-3) Benzimidazole

- **Callis 400 OL (Methyl-thiophante) – Table 6; figure 11**

For methyl-thiophanate, no resistant strains to this fungicide were detected whatever the method used.

The sensitivity to methyl-thiophanate remains good in Ekona farm, situation comparable with previous data.

ii-4) Conclusions and recommendations for Ekona farm

The sensitivity of the oldest used triazole (propiconazole) remains very low on this farm and despite this fungicide is not more used, the use of other fungicides of this same family contributes to a gradual erosion of sensitivity to propiconazole, and in a lower proportion for epoxyconazole. Nevertheless, the sensitivity to difenoconazole and to bitertanol remains similar to the baseline sample.

Frac recommends to apply not more than 8 triazoles per year which include the following products: propiconazole, tebuconazole, bitertanol, difenoconazole, epoxyconazole. Because of the shift observed on propiconazole and epoxyconazole in some sectors, we recommend to reduce this maximum number to 5 applications. By another hand, we recommend also to reinforce deleafing management programs in this plantation because high population levels can accelerate the selection of resistant strains inside the farm.

Strobilurines

The sensitivity of azoxystrobine is close to the baseline sample. But analyses should be opened to many sectors, to carry out the good overview of this plantation. We recommend continuing to follow the current strategy according to Frac recommendation:

-Apply QoI fungicides only in mixtures or alternation with other, non-cross resistant modes of action, all partners at recommended effective manufactor's rates; or. No consecutive QoIs-applications can be applied.

-A maximum of 1 to 2 applications containing QoI fungicides.

-Applications containing QoI fungicides should preferably start at the onset of the annual disease progress curve and be applied at times of lower disease pressure.

-Applications have to be separated by at least 3 months of a QoI-free period.

Benzimidazoles

The sensitivity to methyl-thiophanate is close to the baseline. But analyses should be opened to many sectors to carry out the real situation in this plantation. We recommend the following of the current strategies according to last Frac's recommendations (2010): Benzimidazole fungicides should be applied according to the following guidelines against black sigatoka in banana:

-BCM fungicides have to be applied only in mixtures or in full alternation with other, non-cross resistant modes of action. No consecutive BCM-applications (blocks) can be applied.
- A maximum of 3 applications containing BCM fungicides or a maximum of 33% of the total number of sprays can be applied with BCMs.
- Applications containing BCM fungicides should preferably start at the onset of the annual disease progress curve and be applied at times of lower disease pressure.
- Applications have to be separated by at least 3 months of a BCM-free period.

<u>Table 1</u>: Summary of results concerning the sensitivity of *M. fijiensis* populations to SICO (0.1 ppm of difenoconazole).

Sico	**% inhibition**	**Lenght of the germ tubes (µm)**	**Growth inhibition classes**						**% spores inhibition < 50 %**
Sectors			**0-10**	**11-30**	**31-50**	**51-70**	**71-90**	**91-100**	
Ekona G8/9	**81**	71	0	0	0	0	100	0	**0**
Ekona J6	**73**	87	0	0	0	38	62	0	**0**
Mean	**77**	**79**	**0**	**0**	**0**	**19**	**81**	**0**	**0**
Baseline sample	**81**	**59**	**0**	**0**	**0**	**0**	**100**	**0**	**0**
history									
june-09	79	76	0	0	0	5	95	0	0
oct-09	**77**	**79**	**0**	**0**	**0**	**19**	**81**	**0**	**0**

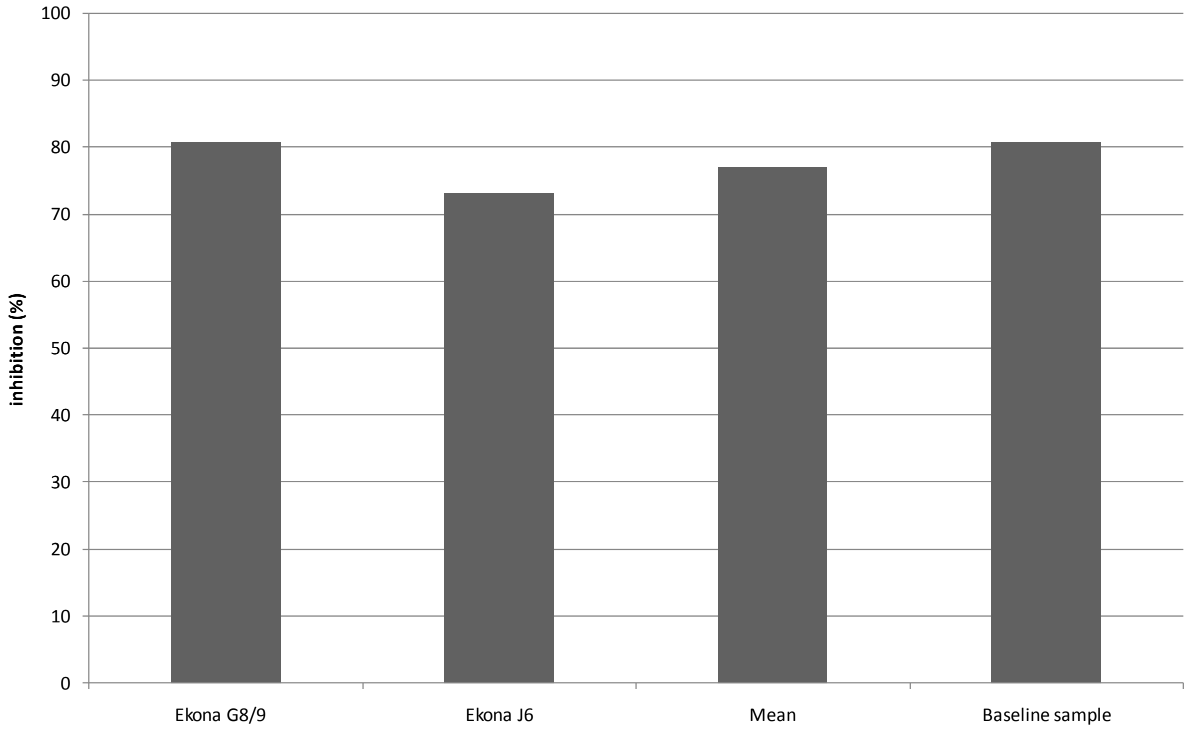


Figure 1: Percentage of growth inhibition (difenoconazole at 0.1 ppm)

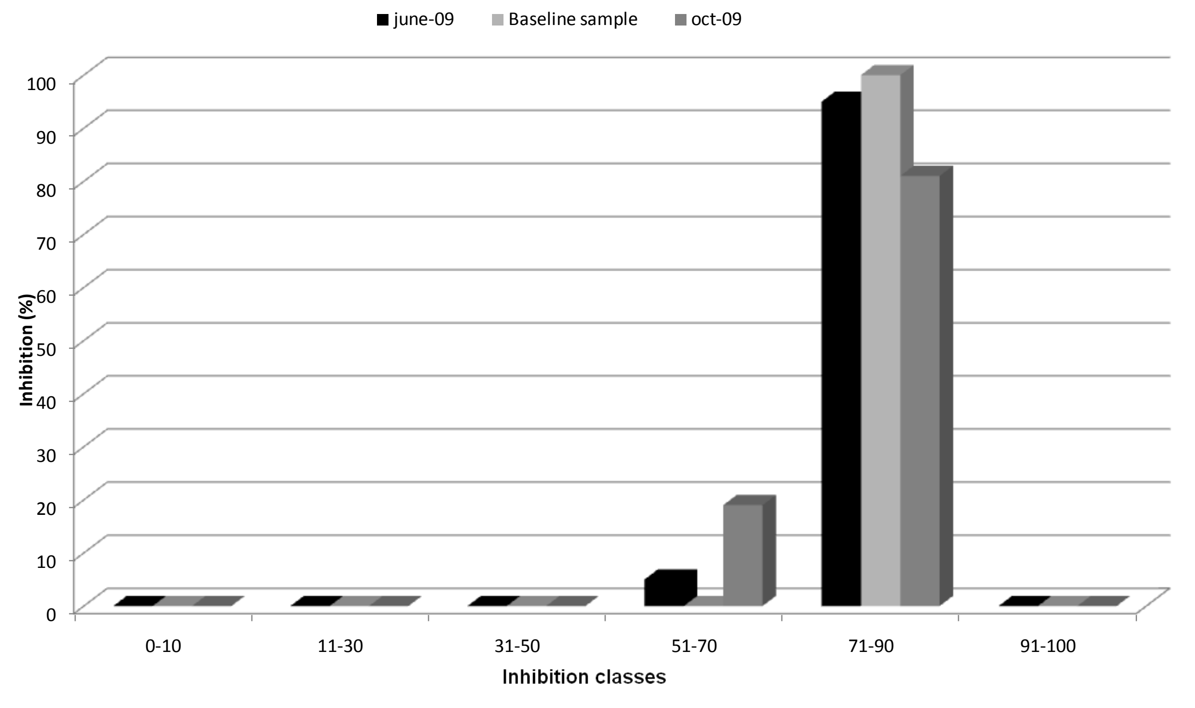


Figure 2: Distribution of spores in inhiition classes (difenoconazole at 0.1 ppm)

<u>Table 2</u> :. **Summary of results concerning the sensitivity of *M. fijiensis* populations to BAYCOR (0.1 ppm of bitertanol).**

BAYCOR	% inhibition	Lenght of the germ tubes (µm)	Growth inhibition classes						% spores inhibition > 50 %
Sectors			0-10	11-30	31-50	51-70	71-90	91-100	
Ekona G8/9	**80**	74	0	0	0	2	98	0	**0**
Ekona J6	**79**	67	0	0	0	14	82	4	**0**
Mean	**80**	**70**	**0**	**0**	**0**	**8**	**90**	**2**	**0**
Baseline sample	**78**	**67**	**0**	**0**	**0**	**0**	**100**	**0**	**0**
History									
déc-05	**83**	39	0	0	0	2	98	0	**0**
dec-06	**74**	63	0	1	17	28	14	40	**18**
nov-07	**70**	72	0	0	0	41	59	0	**0**
june-09	76	84	0	0	1	17	82	0	1
oct-09	**80**	**70**	**0**	**0**	**0**	**8**	**90**	**2**	**0**

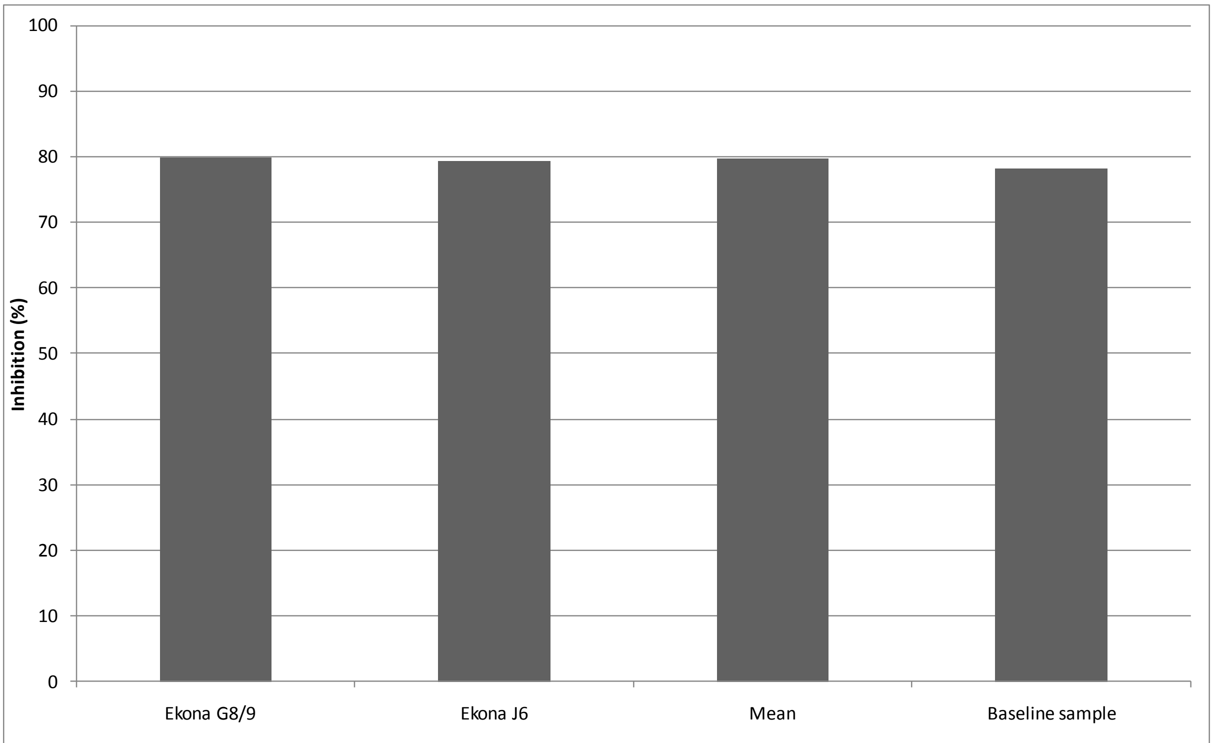


Figure 3: Percentage of inhibition (bitertanol at 0.1 ppm)

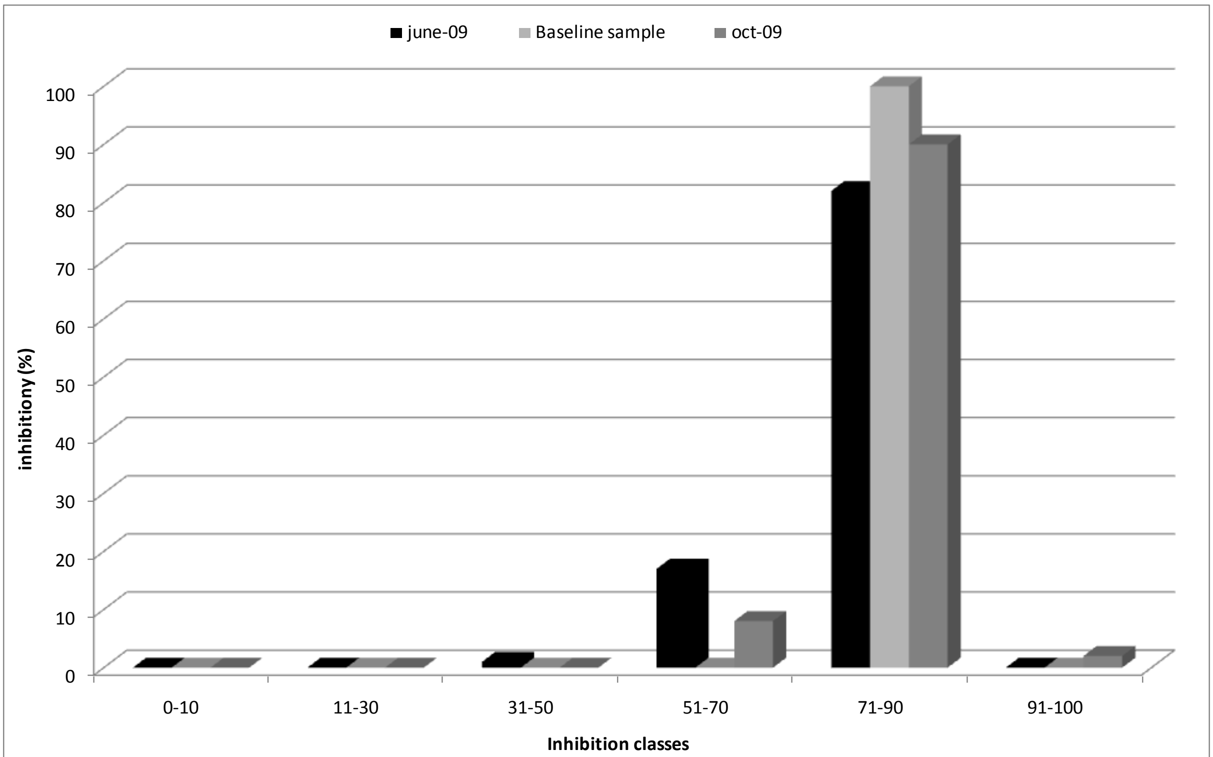


Figure 4: distribution of spores in growth inhibition classes (bitertanol at 0.1 ppm)

<u>Table 3</u>: **Summary of results concerning the sensitivity of *M. fijiensis* populations to OPAL (0.1 ppm of epoxyconazole).**

Opal	**% inhibition**	**Lenght of the germ tubes (µm)**	**Growth inhibition classes**						**% spores inhibition < 50 %**
Sectors			**0-10**	**11-30**	**31-50**	**51-70**	**71-90**	**91-100**	
Ekona G8/9	**70**	109	0	8	8	10	74	0	**16**
Ekona J6	**61**	126	0	4	24	30	42	0	**28**
Mean	**66**	**117**	**0**	**6**	**16**	**20**	**58**	**0**	**22**
Baseline sample	**79**	**65**	**0**	**0**	**0**	**0**	**100**	**0**	**0**
history									
june-09	76	86	0	0	4	20	76	0	4
oct-09	66	117	0	6	16	20	58	0	**22**

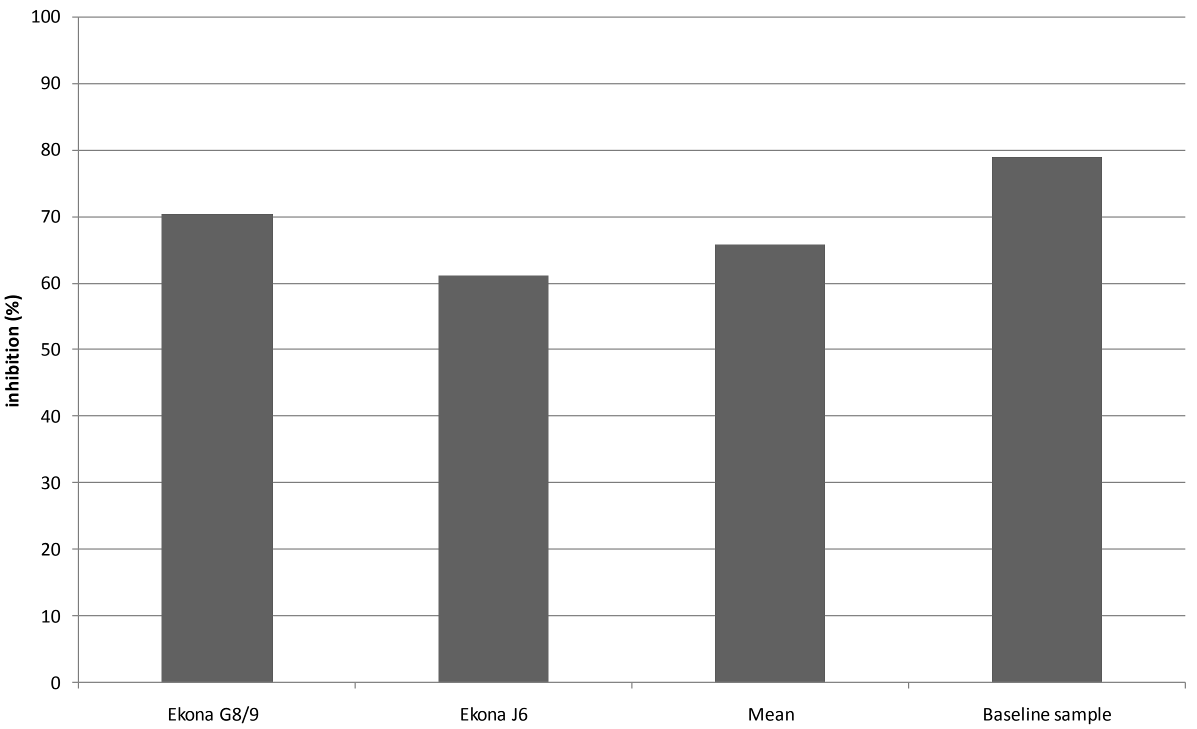


Figure 5: Percentage of growth inhibition (epoxyconazole at 0.1 ppm)

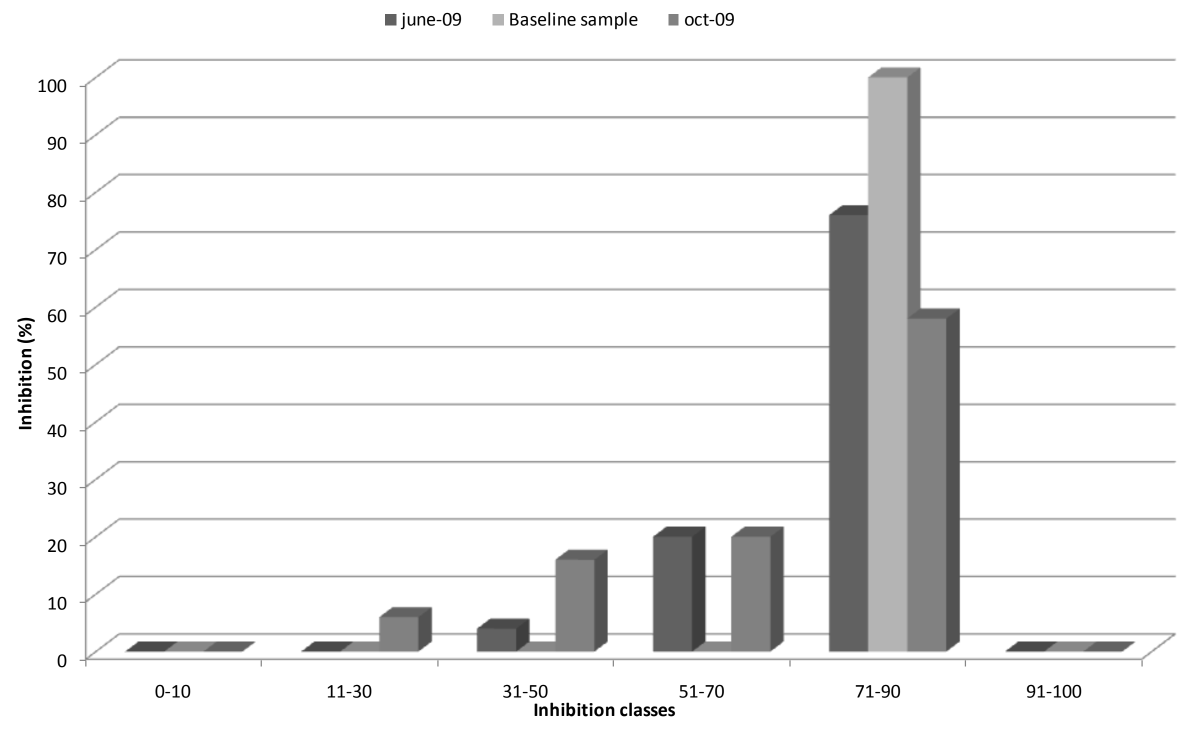


Figure 6: Distribution of spores in growth inhibition (epoxyconazole at 0.1 ppm)

Table 4: Summary of results concerning the sensitivity of *M. fijiensis* populations to TILT (0.1 ppm of propiconazole).

Tilt	% inhibition	Lenght of the germ tubes (µm)	Growth inhibition classes						% spores inhibition < 50 %
Sectors			0-10	11-30	31-50	51-70	71-90	91-100	
Ekona (asco R1)	40	184	4	22	42	20	12	0	**68**
Ekona (asco R2)	47	172	6	18	22	32	22	0	**46**
Ekona (asco R3)	44	197	0	20	50	24	6	0	**70**
Ekona (conidia R1)	25	171	21	32	25	11	0	11	**79**
Ekona (conidia R2)	14	224	44	38	12	3	3	0	**94**
Ekona (conidia R3)	33	169	13	29	32	19	6	0	**74**
mean asco	44	184	3	20	38	25	13	0	**61**
mean conidia	24	188	26	33	23	11	3	4	**82**
total Mean	**34**	**186**	**15**	**27**	**31**	**18**	**8**	**2**	**72**
Baseline sample	**76**	**67**	**0**	**0**	**0**	**22**	**78**	**0**	**0**
History									
may-01	52	-	0	0	49	50	1	0	51
nov-01	59	-	0	3	29	48	20	0	68
july-01	48		0	12	52	29	7	0	36
dec-02	61	-	0	0	19	53	28	0	81
may-04	61	94	0	0	23	58	19	0	77
march-05	68	69	0	0	1	55	42	1	99
déc-05	58	93	0	0	26	60	14	0	74
dec-06	51	121	1	3	45	47	4	0	51
nov-07	55	110	0	4	21	66	9	0	75
oct-09	**34**	**186**	**15**	**27**	**31**	**18**	**8**	**2**	**72**

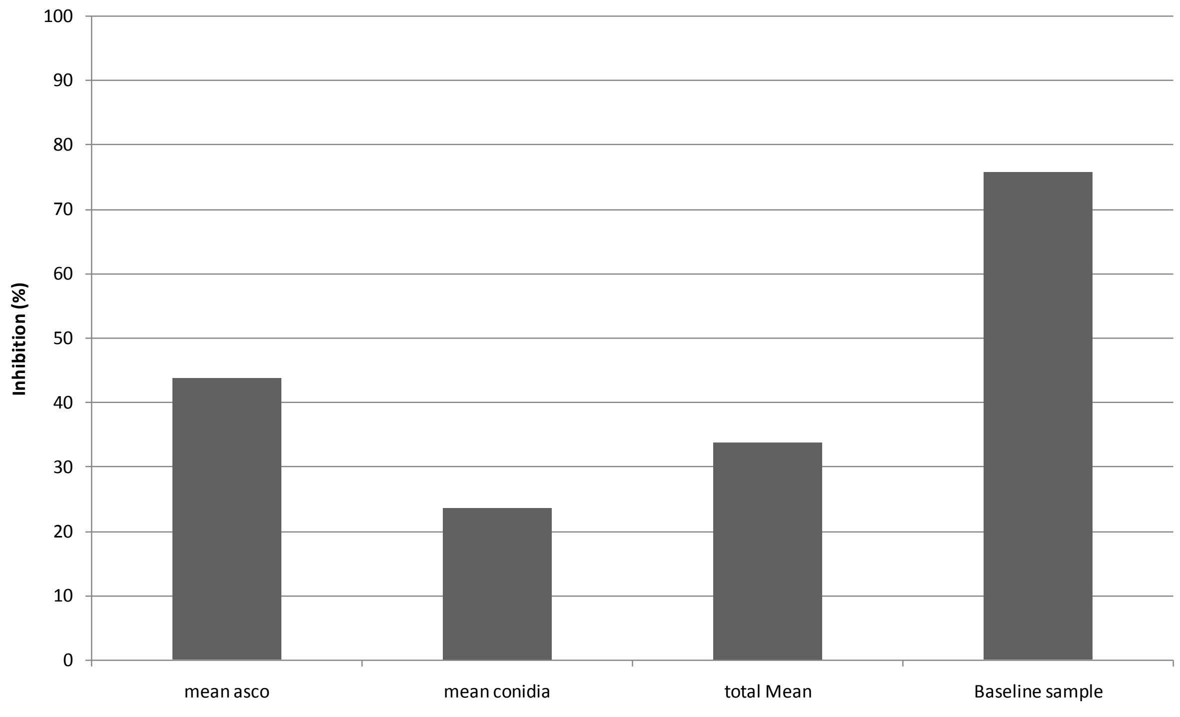


Figure 7: Percentage of growth inhibition (propiconazole at 0.1 ppm)

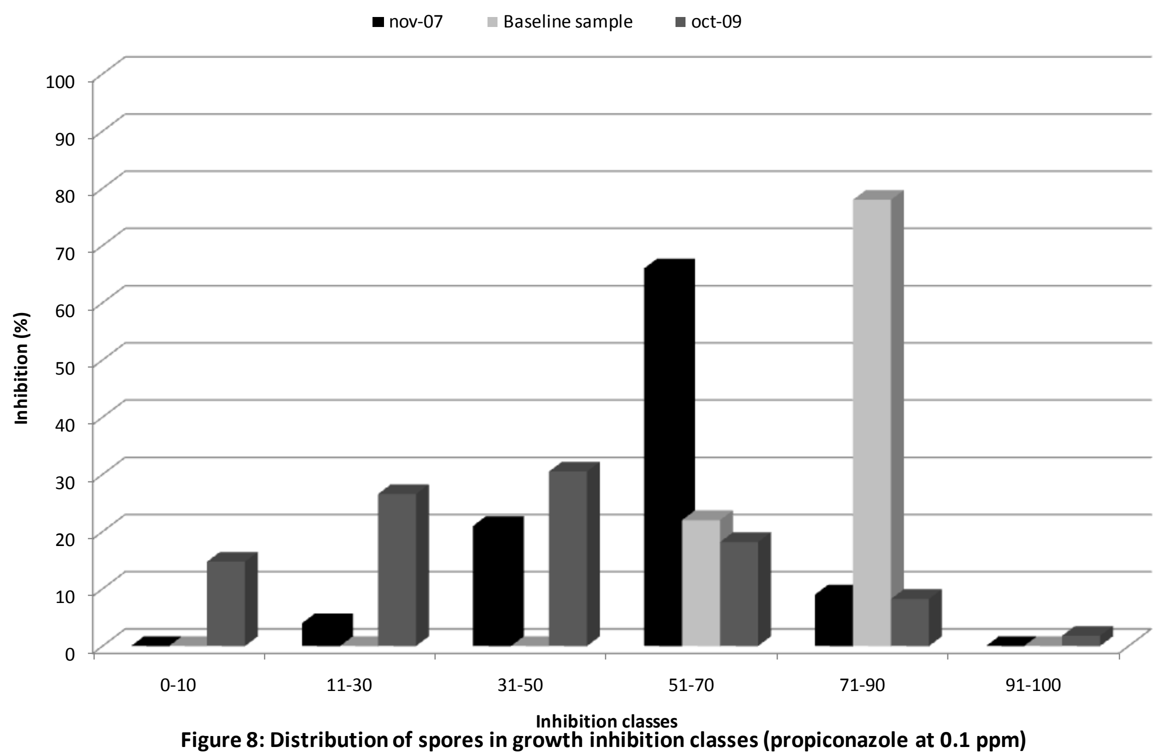


Figure 8: Distribution of spores in growth inhibition classes (propiconazole at 0.1 ppm)

Table 5: Summary of results concerning the sensitivity of *M. fijiensis* populations to BANKIT (10 ppm of azoxystrobin).

Bankit	% inhibition	Lenght of the germ tubes (µm)	Growth inhibition classes						% spores inhibition < 50 %
Sectors			0-10	11-30	31-50	51-70	71-90	91-100	
Ekona (asco R1)	100	0	0	0	0	0	0	100	**0**
Ekona (asco R2)	100	0	0	0	0	0	0	100	**0**
Ekona (asco R3)	100	0	0	0	0	0	0	100	**0**
Ekona (conidia R1)	100	0	0	0	0	0	0	100	**0**
Ekona (conidia R1)	99	3	0	0	0	0	7	93	**0**
Ekona (conidia R1)	97	7	0	0	0	3	13	84	**0**
									0
mean asco	100	0	0	0	0	0	0	100	**0**
mean conidia	98	3	0	0	0	1	7	92	**0**
total Mean	**99**	**2**	**0**	**0**	**0**	**1**	**3**	**96**	**0**
Baseline sample	**100**	**0**	**0**	**0**	**0**	**0**	**0**	**100**	**0**
Summary of the results of previous monitoring									
nov-01	**86**	-	0	0	0	12	88	0	**0**
dec-02	**94**	-	0	0	0	0	50	50	**0**
may-04	**89**	32	0	0	2	8	46	44	**2**
march-05	**92**	33	1	1	1	1	29	67	**3**
dec-05	**89**	37	0	0	0	0	60	40	**0**
oct-09	**99**	**2**	**0**	**0**	**0**	**1**	**3**	**96**	**0**

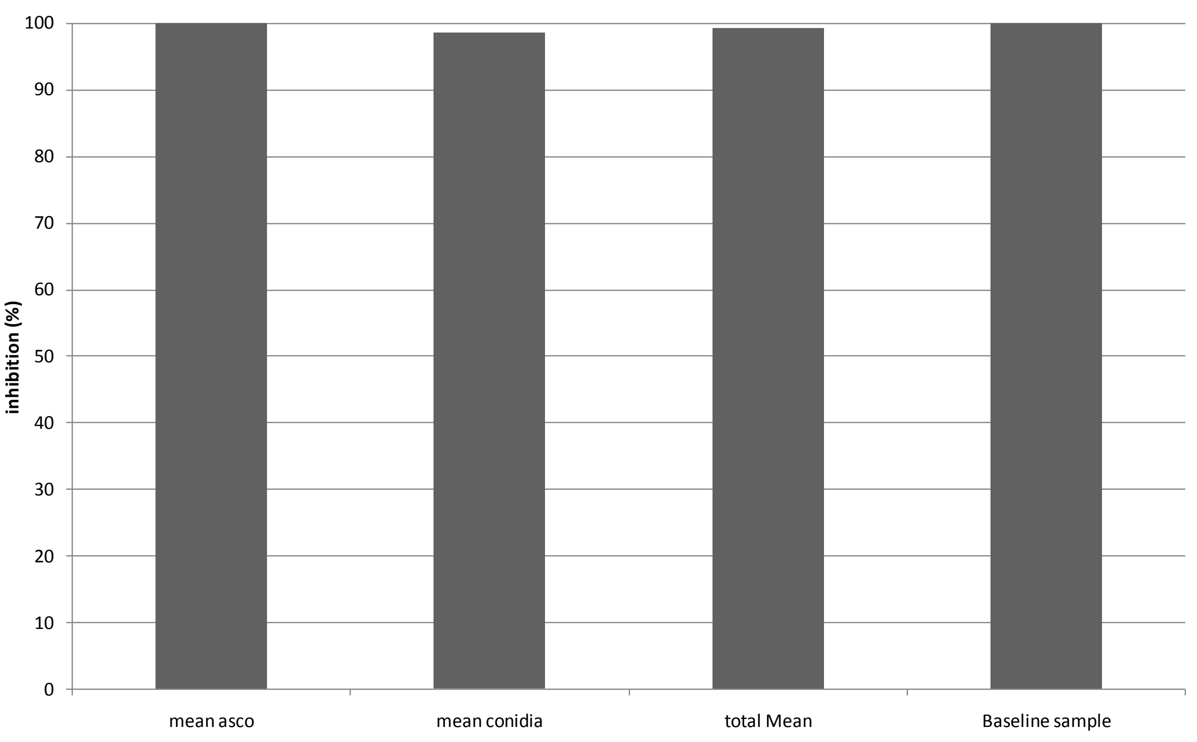


Figure 9: Percentage of growth inhibition (azoxystrobine at 10 ppm)

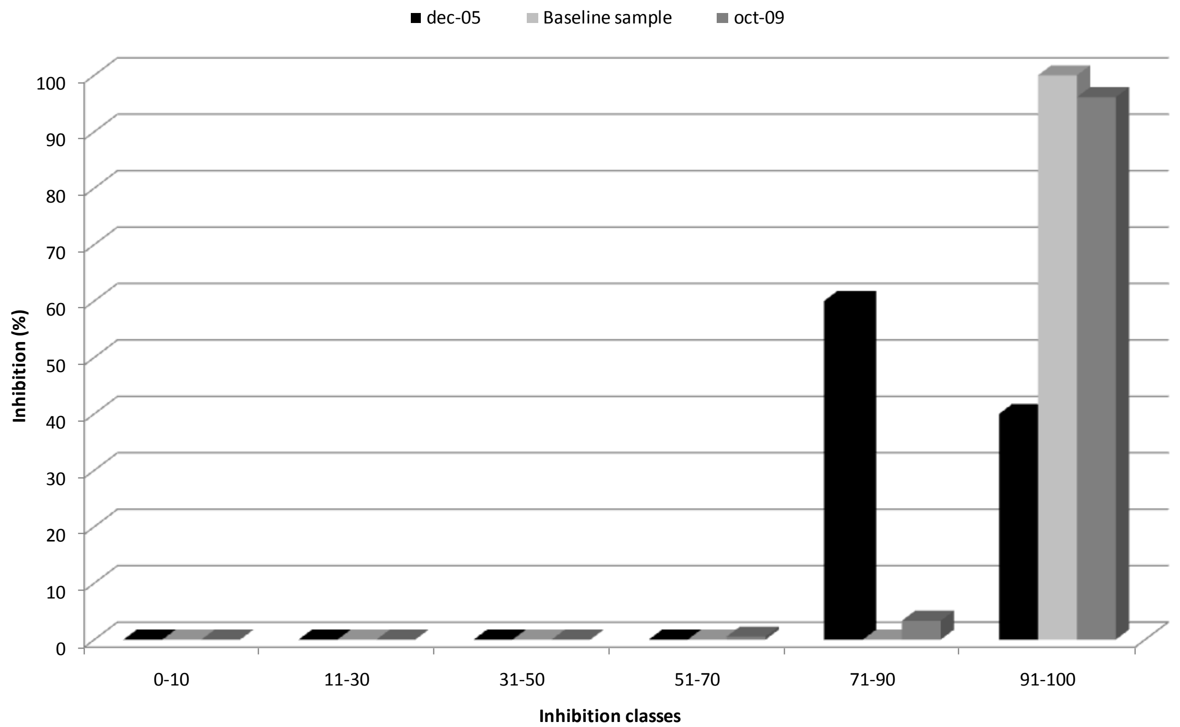


Figure 10: Distribution of spores in growth inhibition classes (azoxystrobine at 10 ppm)

<u>Table 6</u>: Summary of results concerning the sensitivity of *M. fijiensis* populations to CALLIS (5 ppm of methyl-thiophanate).

CALLIS 400 OL Secteurs	Normal	Disturbed	Short Inhibited	None	% sensitive strains	% resistant strains
Ekona (asco R1)	0	95	0	5	**100**	**0**
Ekona (asco R2)	0	99	0	1	**100**	**0**
Ekona (asco R3)	0	100	0	0	**100**	**0**
Ekona (conidia R1)	0	97	0	3	**100**	**0**
Ekona (conidia R1)	0	94	0	6	**100**	**0**
Ekona (conidia R1)	0	97	0	3	**100**	**0**
mean asco	0	98	0	2	**100**	**0**
mean conidia	0	96	0	4	**100**	**0**
total mean	**0**	**97**	**0**	**3**	**100**	**0**
baseline sample	**0**	**100**	**0**	**0**	**100**	**0**
Summary of the results of the previous monitoring						
may-01	0	94	0	6	**100**	**0**
nov-01	0	95	0	5	**100**	**0**
dec-02	0	57	30	13	**70**	**30**
may-04	0	44	15	42	**100**	**0**
March-05	0	100	0	0	**100**	**0**
nov-05	0	89	0	11	**100**	**0**
dec-06	0	100	0	0	**100**	**0**
oct-09	**0**	**97**	**0**	**3**	**100**	**0**

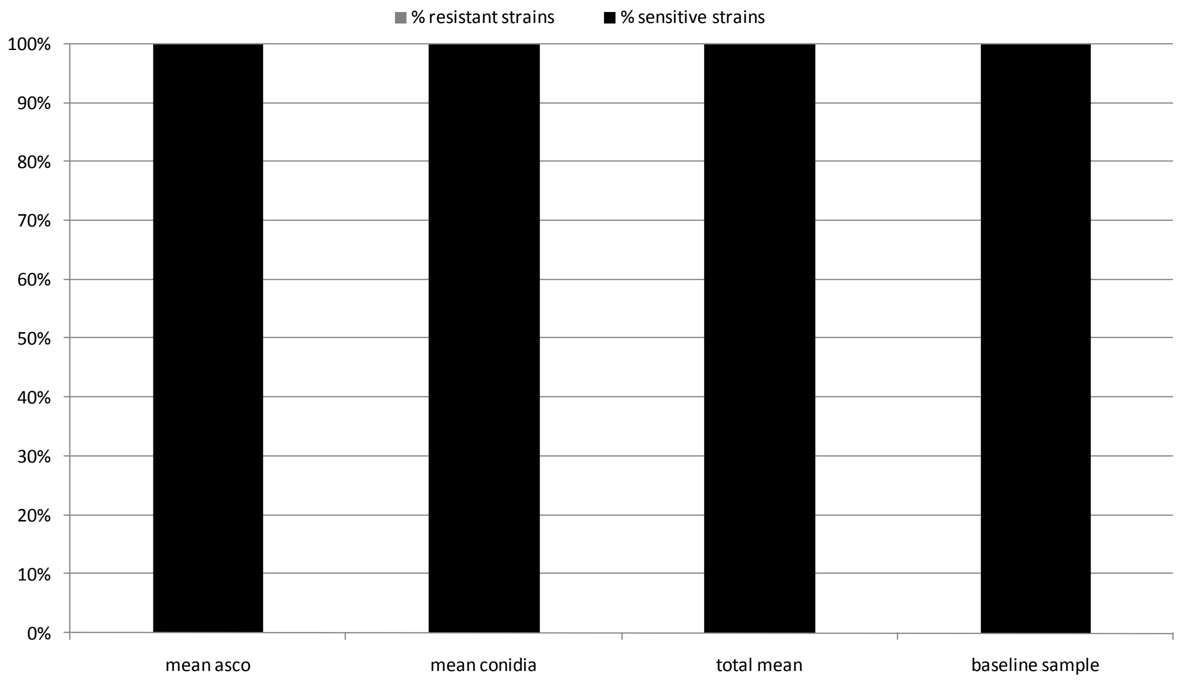


Figure 11: Percentage of sensitive strain (methyl-thophanate at 5 ppm)

iii) Mussaka plantation

iii-1) Triazoles

- ***Sico 250 EC (difenoconazole) – table 1; figures 1 and 2***

For difenoconazole, the average % GI for the 2 different sectors (79-80%) is close to the baseline sample (81%). No strains has a growth inhibition inferior to 50%, phenotype which is not encountered in the baseline sample (100% strains have an inhibition > 50%). This shows no shift in sensitivity to difenoconazole in this plantation. As compared to the previous no change has been observed on this farm.

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- ***Baycor 300 EC (bitertanol) - table 2; figures 3 and 4***

For bitertanol, the average %GI for the 2 different sectors (75-80 %) is similar to the value observed in the baseline sample (78%). A very low unsignificant proportion of the population (0-4%) has a growth inhibition under 50%,. No shift in sensitivity to bitertanol is observed in this plantation. Analyses of previous monitoring show a regular improvement of sensitivity to this fungicide in Mussaka .

- ***Opal 75 EC (epoxyconazole) – table 3; figures 5 and 6***

For epoxyconazole, the average %GI for the 2 different sectors (69-74 %) is close to the baseline sample (79%). A very low proportion of the population (4%) has a growth inhibition under 50%,. No significant shift in sensitivity to epoxyconazole is observed in this plantation. Analyses of the previous monitoring show an improvement of the sensitivity to this fungicide.

- ***Tilt 250 EC (propiconazole) table 4; figures 7 and 8***

For propiconazole, the average % GI for the sector analysed (46-55 51% according to the method) is lower than the percentage on the baseline (76%). An important proportion of the population (40-55 % according to the method) has a growth inhibition < 50%. This shows an important shift in sensivity to propiconazole in Mussaka plantation.

The situation of triazoles is fluctuable. For difenoconazole, bitertanol and epoxyconazole, the level of sensitivity has been improved and is now close to the baseline. Nevertheless, a significant shift of sensitivity to propiconazole was observed during this campaign, showing a gradual erosion of the sensitivity to propiconazole as a consequence of continuous use of products of this family.

iii-2) Strobilurines

- ***Bankit 25 SC (azoxystrobine) - Tableau 5; figures 9 & 10***

For azoxystrobine, very different results have been obtained with the ascospores and the conidial method. The average % GI was significantly lower for the ascospores method (52-54%) as compared to the baseline sample (100%). The difference for the conidial method was not so important (93-95%). With the ascospores method, 18-38 % of the population had a growth inhibition lower than 50 % (100% strains have an inhibition > 50% in the baseline sample); since only a few part of the population (0-7%) had a low growth inhibition with the conidial method.

Even if results are different with the two methods, this is the first time that resistant strains to strobilurines are detected (even at a low level) in this plantation. This situation should be confirmed further

iii-3) Benzimidazole

- ***Callis 400 OL (Methyl-thiophante) – Table 6; figure 11***

For methyl-thiophanate, resistant strains have not been observed. This is confident with the results of previous monitoring.

The sensitivity to methyl-thiophanate closed to the baseline

iii-5) Conclusions and recommendations for Mussaka farm

Triazoles

The global situation of triazoles seems good for most triazoles: difenoconazole, epoxyconazole and bitertanol. Nevertheless, the case of propiconazole for which a strong shift of sensitivity has been observed reveals a continuous erosion of sensitivity as a consequence of triazole use.

Frac recommends to apply not more than 8 triazoles per year which include the following products: propiconazole, tebuconazole, bitertanol, difenoconazole, epoxyconazole. Because of the shift observed on propiconazole in some sectors, we recommend to reduce this maximum number to 5-65 applications of triazole/year. By another hand, we recommend also to reinforce deleafing management programs in this plantation because high population levels can accelerate the selection of resistant strains inside the farm.

Strobilurines

This is the first report of resistant strains to strobilurines in this plantation and a particular attention should be paid to these fungicide. Nevertheless, analyses should be opened to many sectors, to carry out the real situation in this plantation. We recommend to stop temporarily the use of strobilurins in Mussaka and to continue this monitoring in further campaigns.

Benzimidazoles

The sensitivity to methyl-thiophanate is closer to the baseline. But analyses should be opened to many sectors to carry out the real situation in this plantation. We recommend the following of the current strategies according to last Frac's recommendations (2010):

-BCM fungicides have to be applied only in mixtures or in full alternation with other, non-cross resistant modes of action. No consecutive BCM-applications (blocks) can be applied.
- A maximum of 3 applications containing BCM fungicides or a maximum of 33% of the total number of sprays can be applied with BCMs.
- Applications containing BCM fungicides should preferably start at the onset of the annual disease progress curve and be applied at times of lower disease pressure.
- Applications have to be separated by at least 3 months of a BCM-free period.

<u>Table 1:</u> Summary of results concerning the sensitivity of *M. fijiensis* populations to Sico (difenoconazole at 0.1 ppm)

Sico	% inhibition	Lenght of the germ tubes (µm)	Growth inhibition classes						% spores inhibition < 50 %
Sectors			0-10	11-30	31-50	51-70	71-90	91-100	
Mussaka F1C13	**80**	72	0	0	0	2	98	0	**0**
Mussaka F5C19	**79**	63	0	0	0	2	98	0	**0**
Mean	**80**	**68**	**0**	**0**	**0**	**2**	**98**	**0**	**0**
Baseline sample	**81**	**59**	**0**	**0**	**0**	**0**	**100**	**0**	**0**
history									
june-09	**69**	**99**	**0**	**0**	**4**	**41**	**55**	**0**	**4**
nov-09	**80**	**68**	**0**	**0**	**0**	**2**	**98**	**0**	**0**

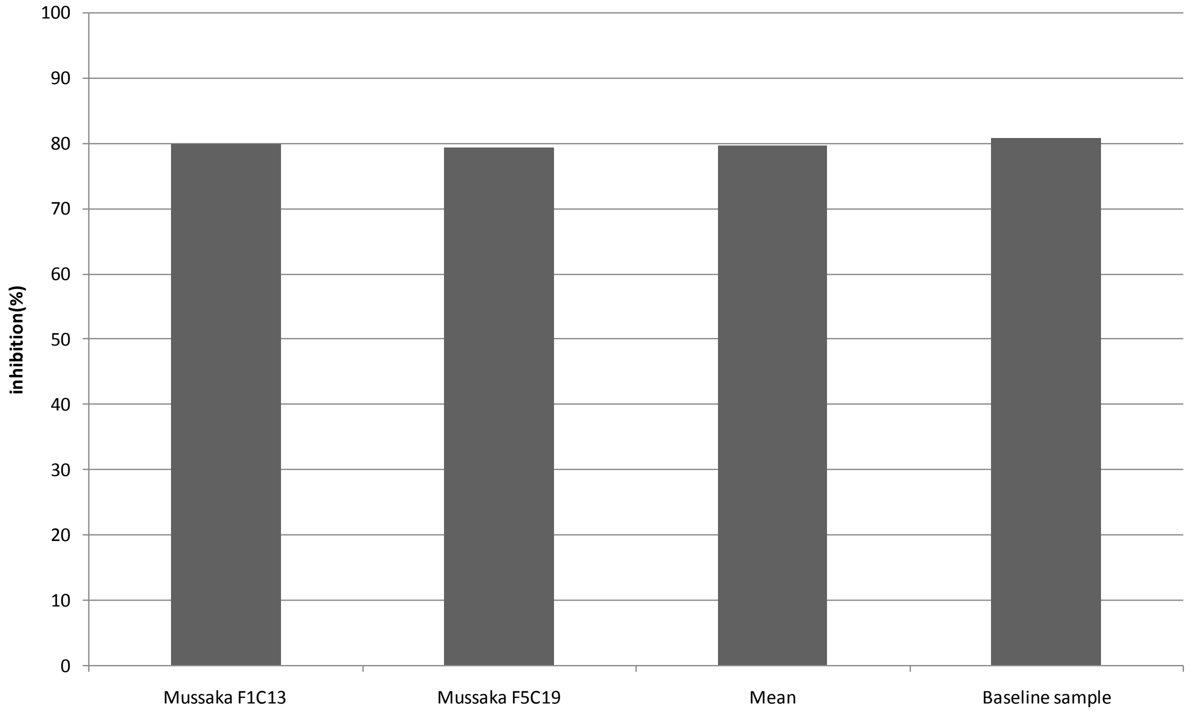

Figure 1: Percentage of growth inhibition (difenoconazole at 0.1 ppm)

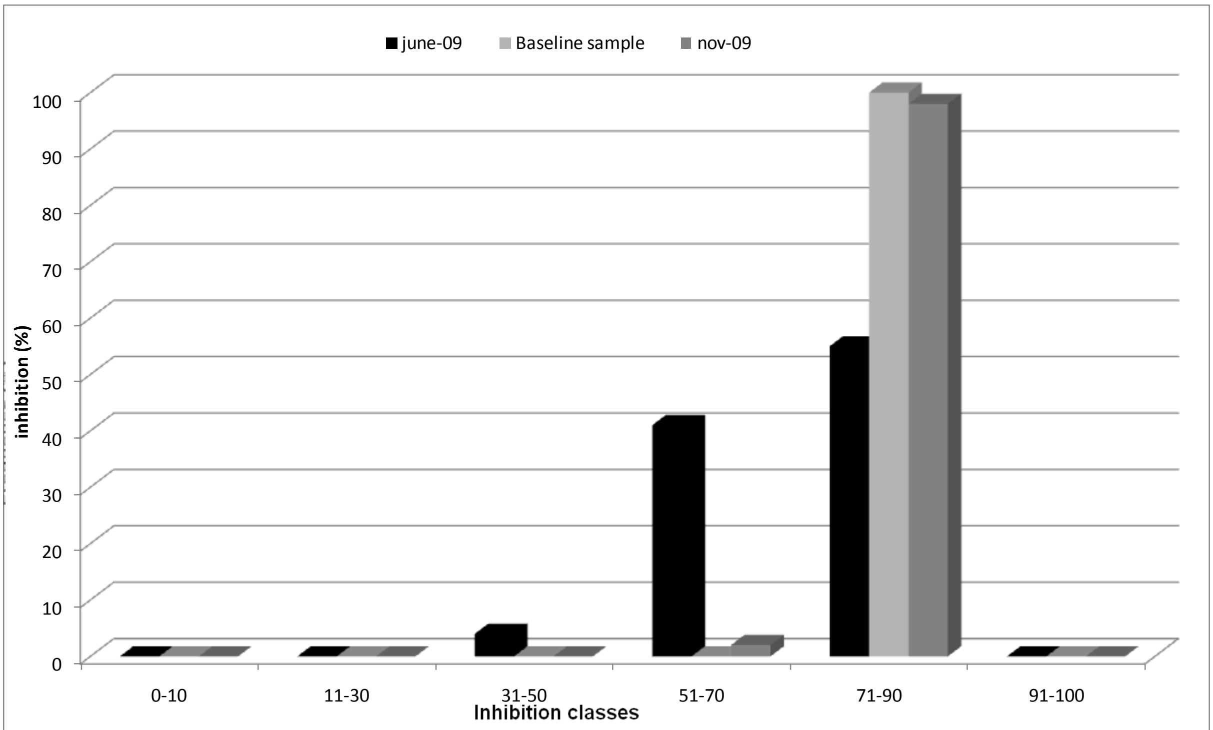

Figure 2: Distribution of spores in inhiition classes (difenoconazole at 0.1 ppm)

<u>Table 2</u>: Summary of results concerning the sensitivity of *M. fijiensis* populations to BAYCOR (bitertanol at 0.1 ppm)

BAYCOR	% inhibition	Lenght of the germ tubes (µm)	Growth inhibition classes						% spores inhibition > 50 %
Sectors			0-10	11-30	31-50	51-70	71-90	91-100	
Mussaka F1C13	**75**	90	0	0	4	18	78	0	**4**
Mussaka F5C19	**80**	60	0	0	0	0	100	0	**0**
Mean	**78**	**75**	**0**	**0**	**2**	**9**	**89**	**0**	**2**
Baseline sample	**78**	**67**	**0**	**0**	**0**	**0**	**100**	**0**	**0**

History

déc-05	**78**	48	0	0	0	9	86	5	**0**
dec-06	**56**	110	0	7	20	59	14	0	**27**
nov-07	**66**	85	0	0	10	44	45	1	**10**
june-09	**67**	106	0	0	14	31	55	0	**14**
nov-09	**78**	**75**	**0**	**0**	**2**	**9**	**89**	**0**	**2**

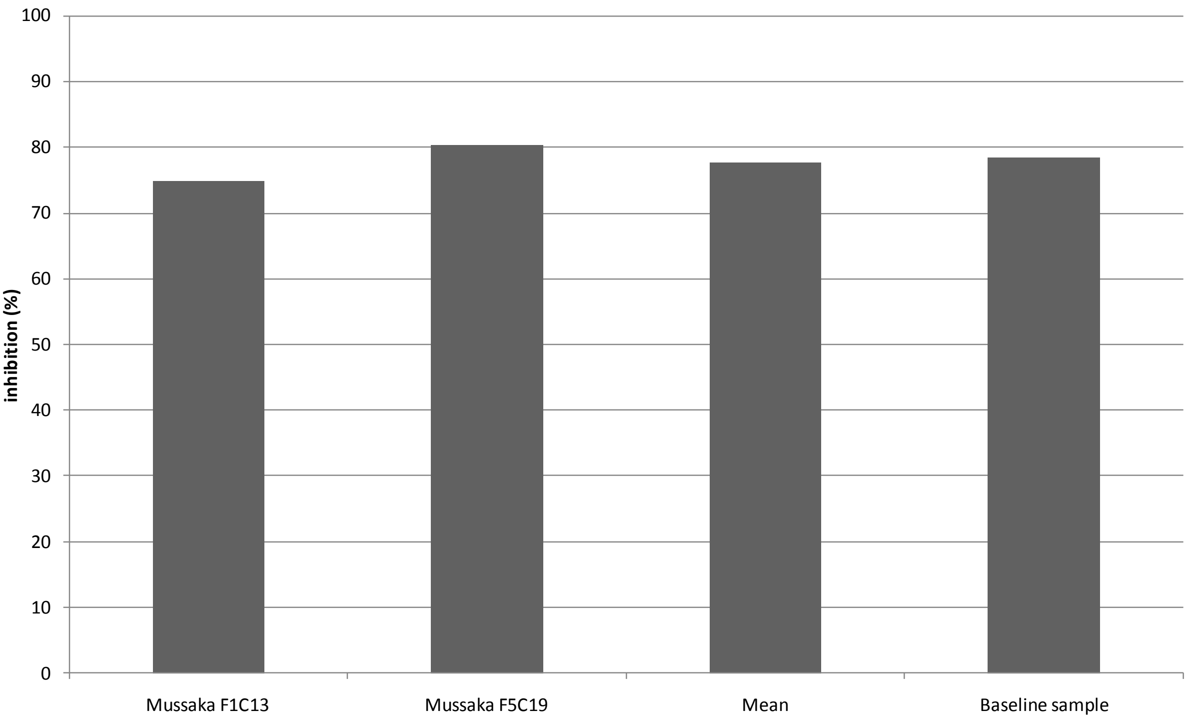

Figure 3: Percentage of inhibition (bitertanol at 0.1 ppm)

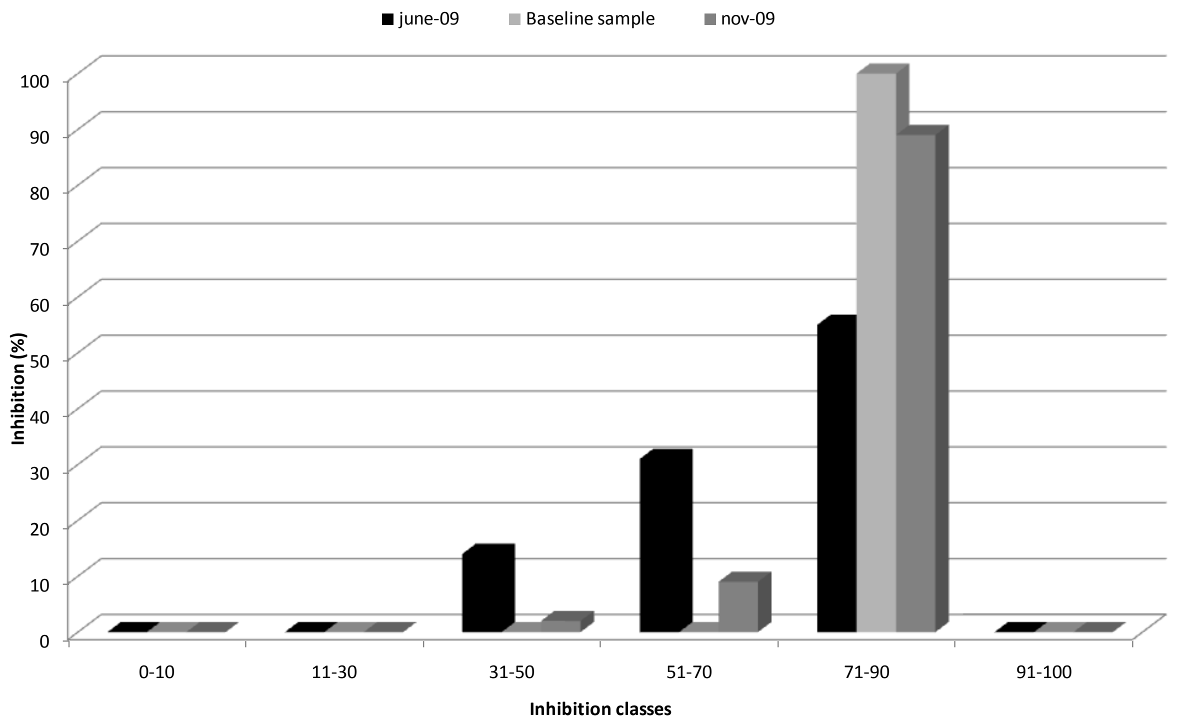

Figure 4: distribution of spores in growth inhibition classes (bitertanol at 0.1 ppm)

<u>Table 3</u>: Summary of results concerning the sensitivity of *M. fijiensis* populations to Opal (epoxyconazole at 0.1 ppm).

Opal	% inhibition	Lenght of the germ tubes (µm)	Growth inhibition classes						% spores inhibition < 50 %
Sectors			0-10	11-30	31-50	51-70	71-90	91-100	
Mussaka F1C13	**74**	94	0	0	4	26	70	0	**4**
Mussaka F5C19	**69**	94	0	0	4	40	56	0	**4**
Mean	**72**	**94**	**0**	**0**	**4**	**33**	**63**	**0**	**4**
Baseline sample	**79**	**65**	**0**	**0**	**0**	**0**	**100**	**0**	**0**

history									
june-09	**61**	123	0	2	27	29	42	0	**29**
nov-09	**72**	**94**	**0**	**0**	**4**	**33**	**63**	**0**	**4**

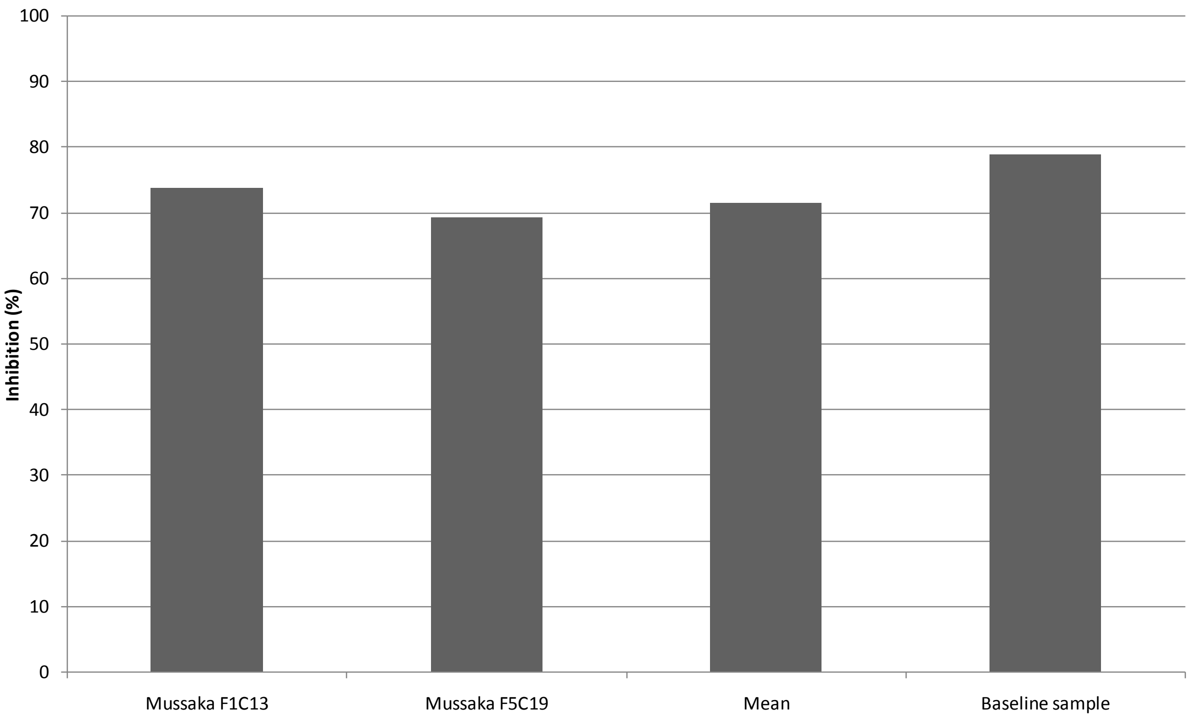

Figure 5: Percentage of growth inhibition (epoxyconazole at 0.1 ppm)

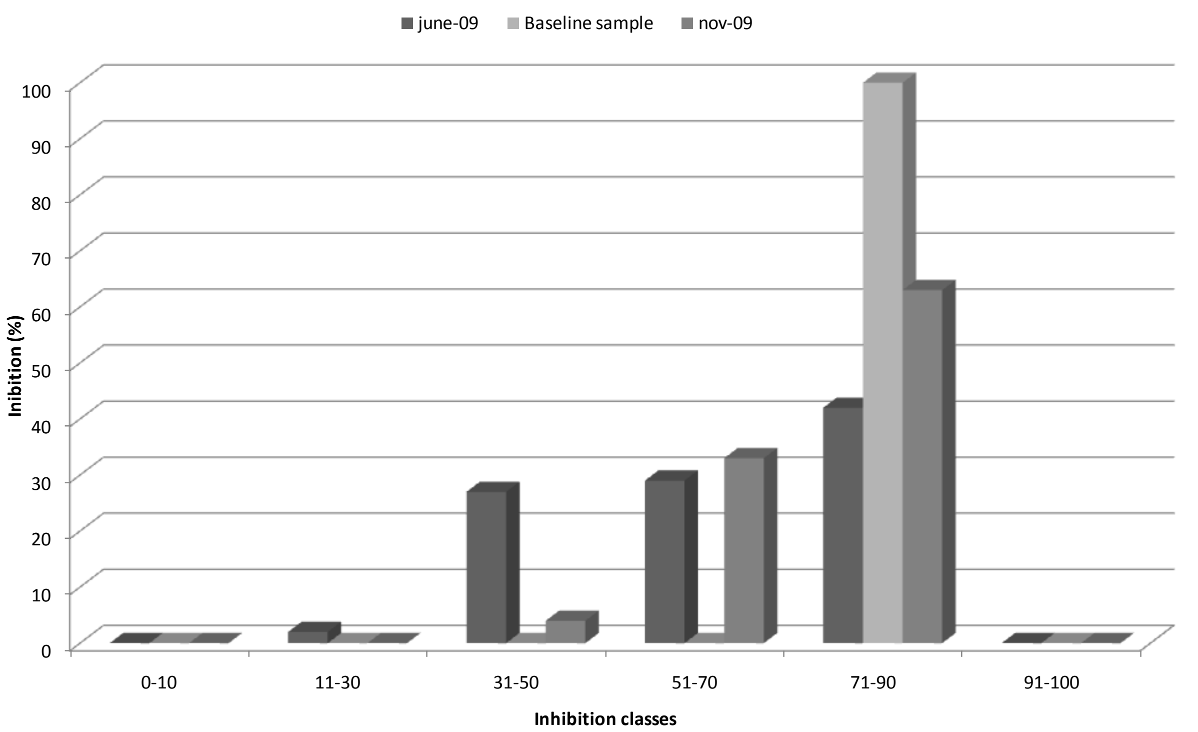

Figure 6: Distribution of spores in growth inhibition (epoxyconazole at 0.1 ppm)

Table 4: **Summary of results concerning the sensitivity of *M. fijiensis* populations to Tilt (propiconazole at 0.1 ppm).**

Tilt	% inhibition	Lenght of the germ tubes (µm)	Growth inhibition classes						% spores inhibition < 50 %
Sectors			0-10	11-30	31-50	51-70	71-90	91-100	
Mussaka (asco r1)	**53**	133	0	8	34	52	6	0	**42**
Mussaka (asco r2)	**52**	140	0	4	56	34	6	0	**60**
Mussaka (asco r3)	**61**	125	0	2	16	54	28	0	**18**
Mussaka (conidie r1)	**53**	104	5	10	32	29	20	5	**46**
Mussaka (conidie r2)	**48**	114	4	10	38	35	6	6	**52**
Mussaka (conidie r3)	**36**	121	12	25	29	24	6	4	**67**
mean asco	55	132	0	5	35	47	13	0	**40**
mean conide	46	113	7	15	33	29	11	5	**55**
Total Mean	**51**	**123**	**3**	**10**	**34**	**38**	**12**	**3**	**48**
Baseline sample	**76**	**67**	**0**	**0**	**0**	**22**	**78**	**0**	**0**
history									
mars-05	**81**	42	0	0	0	6	90	4	**0**
nov-05	**68**	67	0	0	7	33	60	0	**7**
nov-09	**51**	**123**	**3**	**10**	**34**	**38**	**12**	**3**	**48**

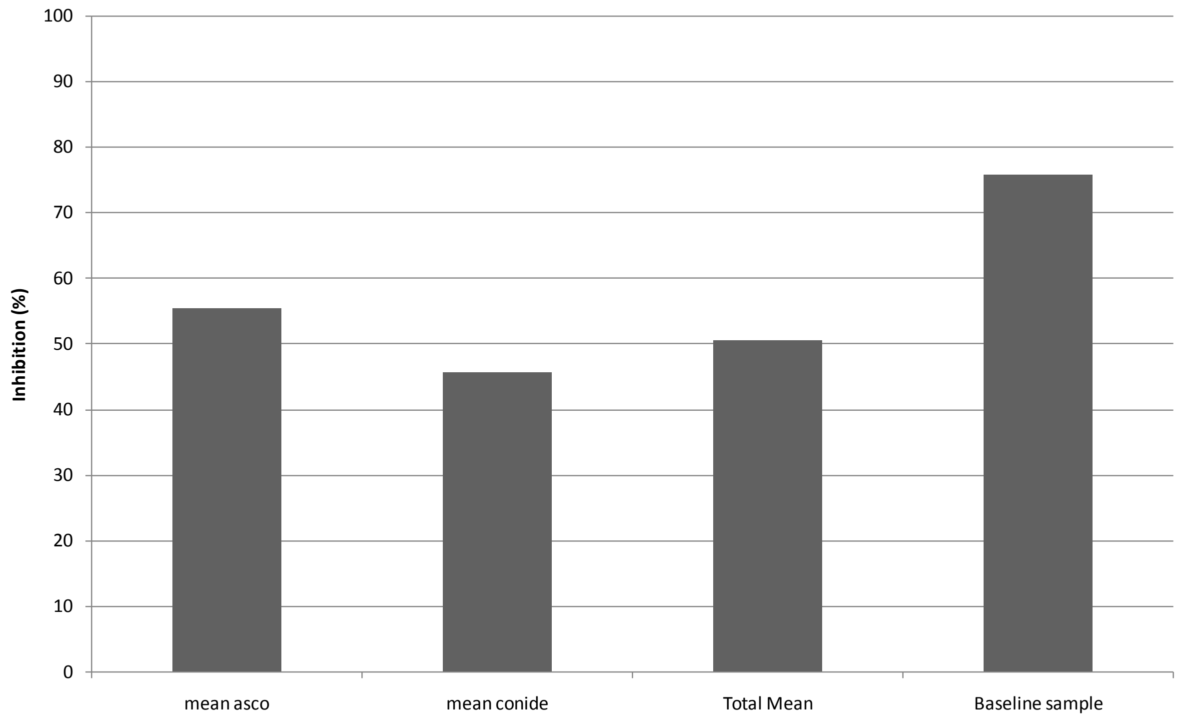

Figure 7: Percentage of growth inhibition (propiconazole at 0.1 ppm)

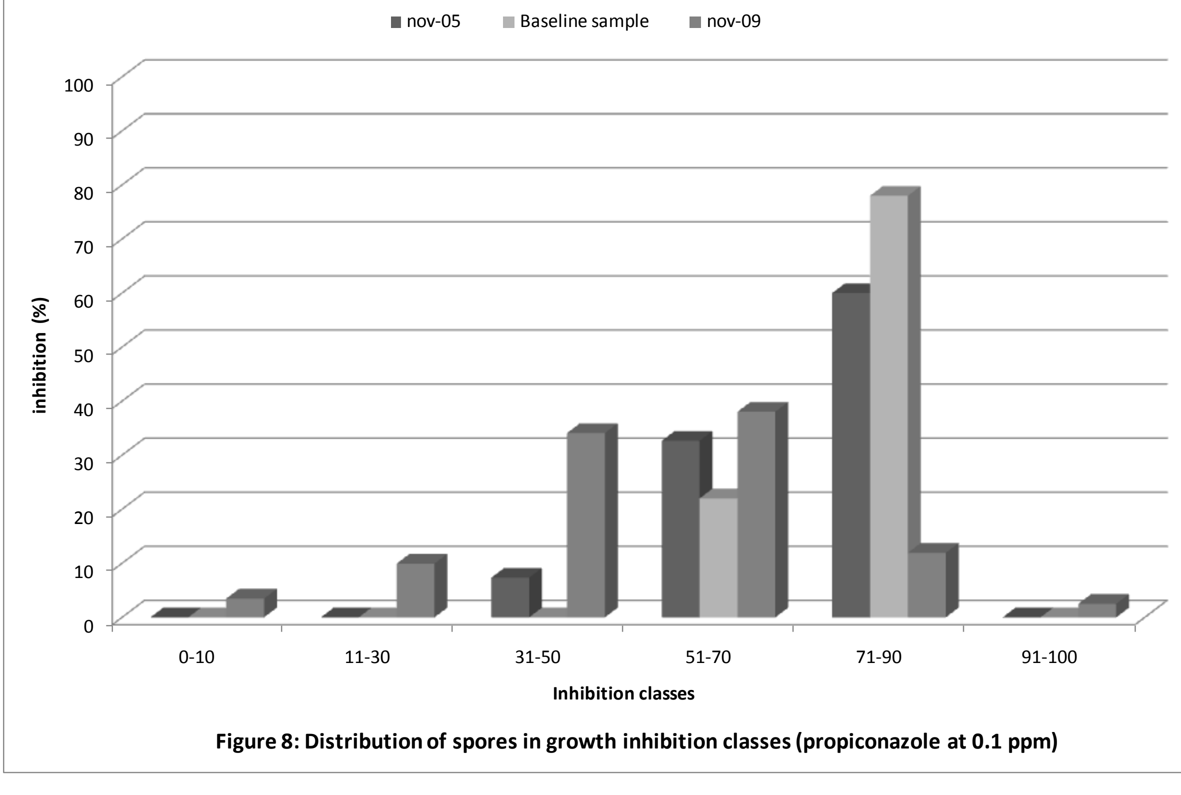

Figure 8: Distribution of spores in growth inhibition classes (propiconazole at 0.1 ppm)

Table 5: Summary of results concerning the sensitivity of *M. fijiensis* populations to Bankit (azoxystrobine at 10 ppm).

Bankit	% inhibition	Lenght of the germ tubes (µm)	Growth inhibition classes						% spores inhibition < 50 %
Sectors			0-10	11-30	31-50	51-70	71-90	91-100	
Mussaka (asco R1)	54	58	0	0	18	26	2	54	**18**
Mussaka (asco R2)	54	69	0	2	36	2	6	54	**38**
Mussaka (asco R3)	52	64	0	0	27	13	2	58	**27**
Mussaka (conidia R1)	93	14	0	0	0	16	2	82	**0**
Mussaka (conidia R2)	94	13	2	0	5	2	0	90	**7**
Mussaka (conidia R3)	95	10	3	0	0	5	0	92	**3**
mean asco	53	64	0	1	27	14	3	55	**28**
mean conidia	94	12	2	0	2	8	1	88	**3**
Total Mean	**74**	**38**	**1**	**0**	**14**	**11**	**2**	**72**	**15**
Baseline sample	**100**	**0**	**0**	**0**	**0**	**0**	**0**	**100**	**0**
history									
march-05	**92**	29	0	0	0	2	41	57	**0**
dec-05	**93**	19	0	0	0	1	39	60	**0**
nov-09	**74**	**38**	**1**	**0**	**14**	**11**	**2**	**72**	**15**

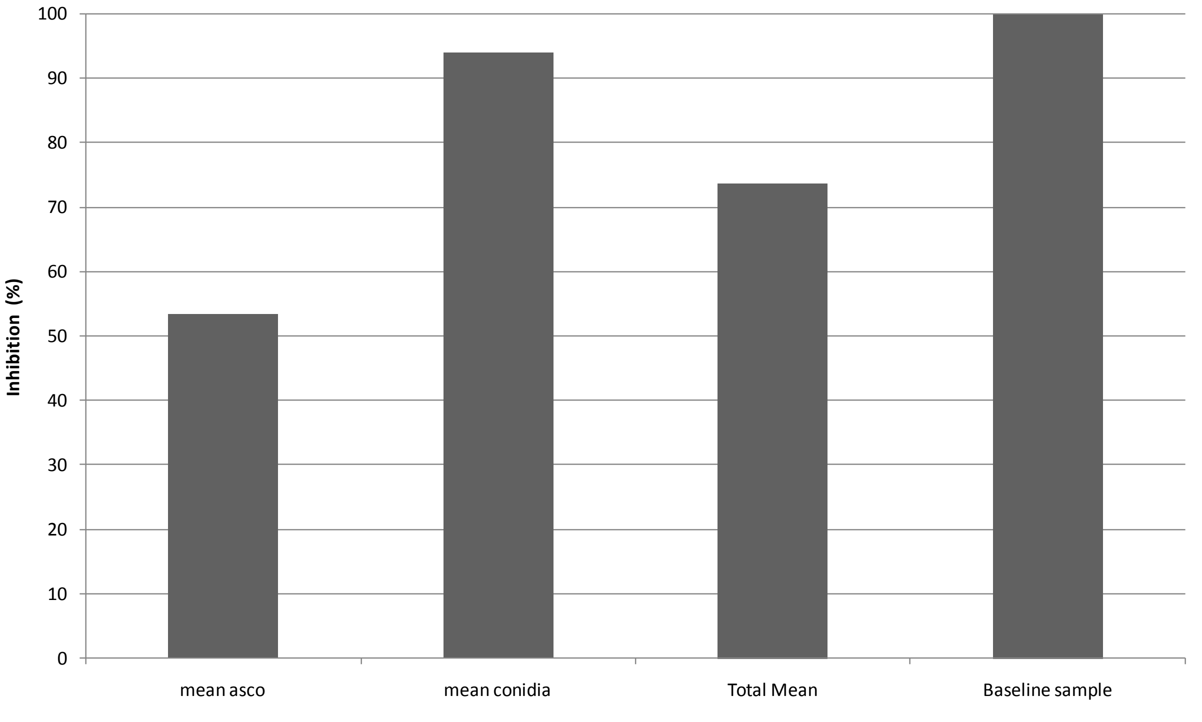

Figure 9: Percentage of growth inhibition (azoxystrobine at 10 ppm)

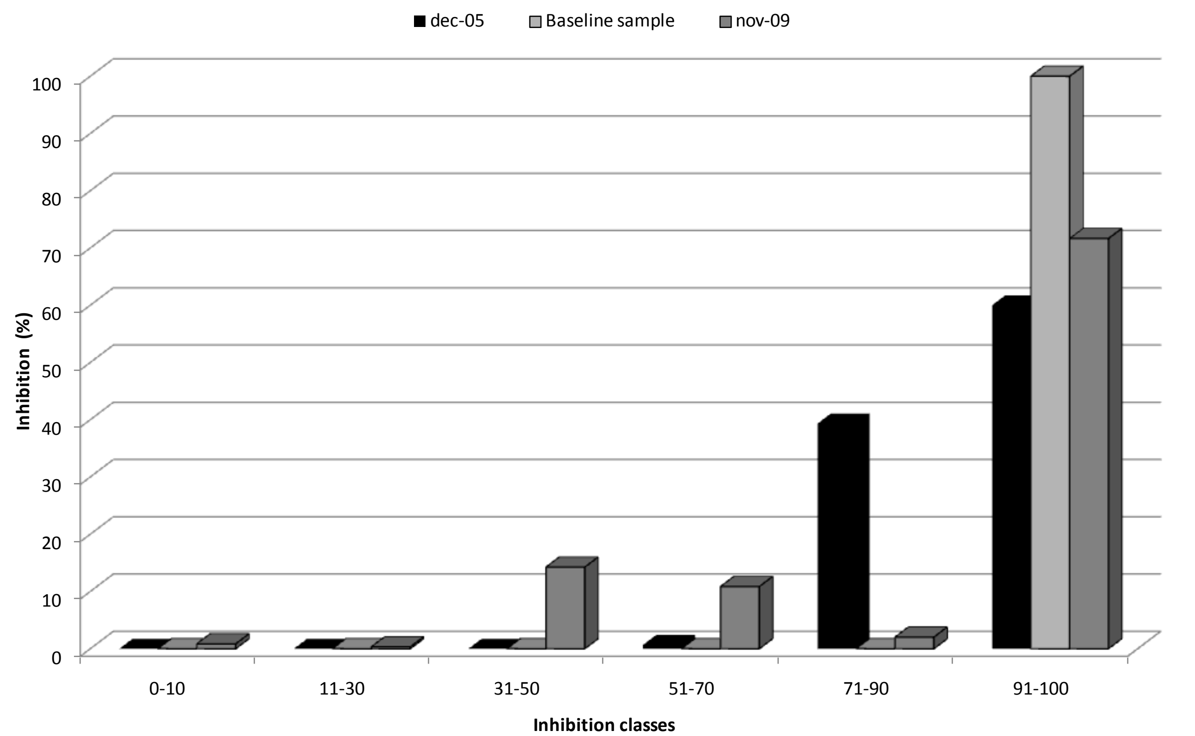

Figure 10: Distribution of spores in growth inhibition classes (azoxystrobine at 10 ppm)

<u>Table 6</u>: Summary of results concerning the sensitivity of *M. fijiensis* populations to Callis (methyl-thiopahante at 5 ppm).

CALLIS 400 OL Secteurs	Normal	Disturbed	Short Inhibited	None	% sensitive strains	% resistant strains
Mussaka (asco R1)	0	100	0	0	**100**	**0**
Mussaka (asco R2)	0	100	0	0	**100**	**0**
Mussaka (asco R3)	0	100	0	0	**100**	**0**
Mussaka (conidia R1)	0	94	0	6	**100**	**0**
Mussaka (conidia R2)	0	68	0	32	**100**	**0**
Mussaka (conidia R3)	0	91	3	6	**97**	**3**
mean asco	0	100	0	0	**100**	**0**
mean conidia	0	84	1	15	**99**	**1**
total mean	**0**	**92**	**1**	**7**	**100**	**1**
baseline sample	**0**	**100**	**0**	**0**	**100**	**0**
history						
mars-05	2	98	0	0	**98**	**2**
nov-05	0	89	3	8	**97**	**3**
dec-06	5	95	0	0	**95**	**5**
nov-09	**0**	**92**	**1**	**7**	**100**	**1**

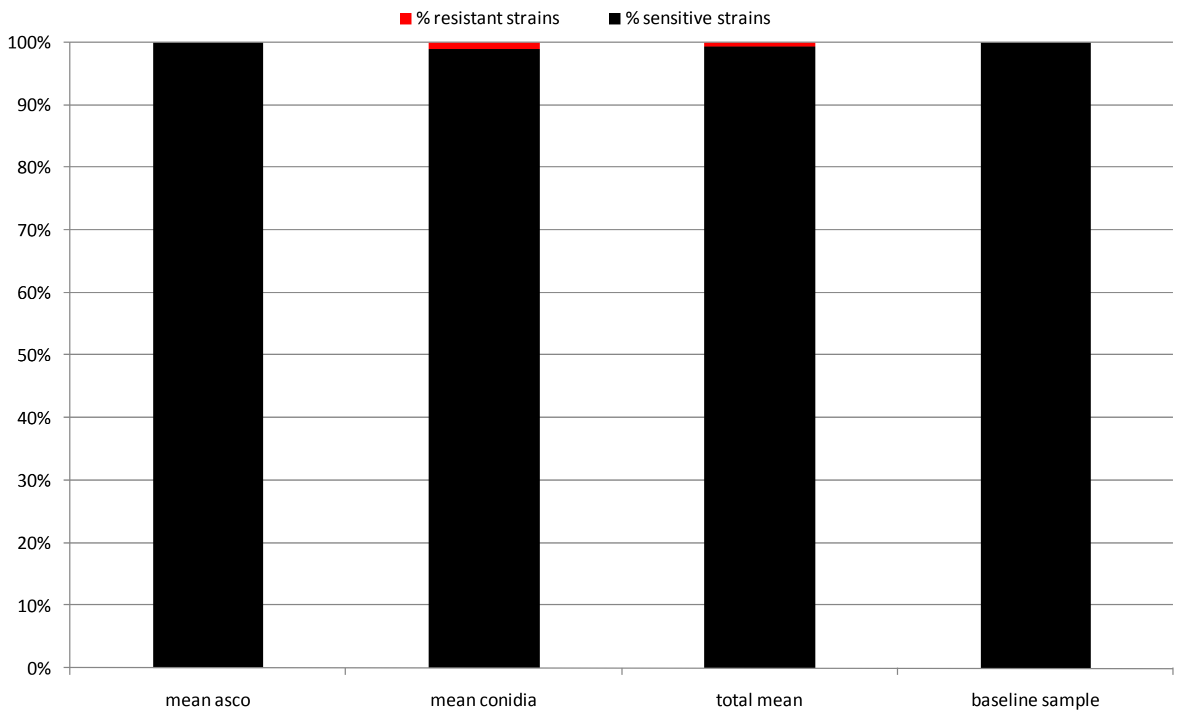

Figure 11: Percentage of sensitive strain (methyl-thophanate at 5 ppm)

For CARBAP

The Director